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BRAIN RESEARCH REVIEWS

Review

Effect of hyperbaric oxygenation on brain hemodynamics, hemoglobin oxygenation and mitochondrial NADH

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ABSTRACT

To determine the HbO_2 oxygenation level at the microcirculation, we used the hyperbaric chamber. The effects of hyperbaric oxygenation (HBO) were tested on vitality parameters in the brain at various pressures. Microcirculatory hemoglobin oxygen saturation (HbO_2), cerebral blood flow (CBF) and mitochondrial NADH redox state were assessed in the brain of awake restrained rats using a fiber optic probe. The hypothesis was that HBO may lead to maximal level in microcirculatory HbO_2 due to the amount of the dissolved O_2 to provide the O_2 consumed by the brain, and therefore no O_2 will be dissociated from the HbO_2. Awake rats were exposed progressively to 15 min normobaric hyperoxia, 100% O_2 (NH) and to 90 min hyperbaric hyperoxia (HH) from 1.75 to 6.0 absolute atmospheres (ATA). NH and HH gradually decreased the blood volume measured by tissue reflectance and NADH but increased HbO_2 in relation to pO_2 in the chamber up to a nearly maximum effect at 2.5 ATA. Two possible approximations were found to describe the relationship between NADH and HbO_2: linear or logarithmic. These findings show that the increase in brain microcirculatory HbO_2 is due to an increase in O_2 supply by dissolved O_2, reaching a maximum at 2.5 ATA. NADH is oxidized (decreased signal) in parallel to the HbO_2 increase, showing maximal tissue oxygenation and cellular mitochondrial NADH oxidation at 2.5 ATA. In conclusion, in the normoxic brain, the level of microcirculatory HbO_2 is about 50% as compared to the maximal level recorded at 2.5 ATA and the minimal level measured during anoxia.

Contents

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1. Introduction

Under normal conditions, most of the oxygen carried in the blood is bound to hemoglobin, which is 98% saturated in the blood leaving the heart at sea level pressure. Some dissolved oxygen is however carried in the plasma, and this portion is proportional to the increase in partial pressure of O_2 according to Henry's Law, maximizing tissue oxygenation (Gill and Bell, 2004). The systemic circulation involves diffusion of O_2 from the capillary bed to the mitochondria which are entirely dependent on the oxygen gradient between the microcirculation and the mitochondria (Tsai et al., 2003).

The main approach to monitor systemic blood oxygenation is by a pulse oximeter, reflecting the HbO_2 saturation in large arteries. This information does not necessarily reflect the oxygenation of blood in the microcirculation.

The steady state level of Hb oxygenation in the microcirculation represents the balance between O_2 consumption by the cells and by tissue blood supply providing new highly oxygenated blood. Our hypothesis was that brain hemoglobin oxygenation, at the microcirculation level, is in the range of 50–60% due to the high O_2 consumption and dissociation of O_2 from the HbO_2 and its diffusion to the mitochondria. The only way to achieve 100% Hb oxygenation in the microcirculation is by exposing the tissue to hyperbaric oxygenation-HBO and supplying the O_2 consumed by the cells by high levels of dissolved O_2. Araki et al. (1988) found that at 2 ATA O_2, hemoglobin in the brain tissue is only 80% saturated. There is no way to sample directly microcirculatory blood and determine the level of Hb saturation.

The metabolic states of the mitochondria defined by Chance and Williams (1955) were only based on *in vitro* studies. They defined state 4 as the resting state (NADH 99% reduced) and state 3 as the active state (NADH 55% reduced) namely, in state 3 the NADH is oxidized. Oxygen balance at the cellular level can be evaluated *in vivo* by NADH fluorometry (Mayevsky, 1984; Mayevsky et al., 2004). Chance et al. (1973) showed in detail that NADH is the most oxygen-sensitive component of the mitochondrial respiratory chain and therefore can best serve as an indicator of intracellular O_2 concentration and mitochondrial activity. The brain *in vivo*, at the resting normoxic state, is in the range of 3.5–3.6 compared to resting mitochondria *in vitro* that are in state 4 (Mayevsky et al., 1974, 2002). While the maximal level of NADH could be determined by exposing the intact brain to pure nitrogen, it is very difficult to measure the minimal level of NADH *in vivo* (maximal level of NAD^+).

The maximal oxidation level of NADH (minimal level of NADH signal) in the brain was determined *in vivo* in the past, under hyperbaric oxygenation (Mayevsky and Shaya, 1980). Since the monitoring of NADH redox state provides relative units, the range between maximal and minimal fluorescence level is of a significant value. Oxygen supply to the cells is dependent also on the level of Hb oxygenation; therefore, it is important to identify its levels in the microcirculation. We suggest that with a rise in the hyperbaric chamber O_2 pressure, hemoglobin saturation in the brain tissue increases. When there is no additional increase, the hemoglobin in the brain is fully saturated (similar to the systemic values) and its level can be compared to the maximal changes in NADH oxidation. The minimal level of microcirculatory HbO_2 is measured by exposing the rat to 100% N_2, which induce complete desaturation of HbO_2 and maximal levels of NADH.

In our laboratory, we developed an optical multiparametric monitoring system, a laser Doppler flowmeter (LDF) to measure CBF, and an *in vivo* 2 wavelengths reflectometer to measure microcirculatory HbO_2 (Rampil et al., 1992). These are two parameters that can provide data on the microcirculatory oxygen transport level. In order to evaluate oxygen availability at the cellular level, we used mitochondrial NADH fluorometry *in vivo* (Mayevsky, 1978a; Mayevsky et al., 2002, 2004, 1998). The hypothesis was that HBO may lead to maximal level in microcirculatory HbO_2 due to the capacity of the dissolved O_2 to provide the O_2 consumed by the brain, and therefore no O_2 will be dissociated from the HbO_2. Under hyperbaric oxygenation conditions the main source for cellular O_2 is the increased dissolved oxygen replacing the delivery by the desaturation of oxygenated hemoglobin, and therefore the HbO_2 will be maximally oxygenated.

The aims of this study were as follows: (1) to determine the HBO level at which *in vivo* hemoglobin oxygenation in brain microcirculation, is maximal; (2) to find the relation between those values to the real time mitochondrial NADH redox state; and (3) to compare the responses of CBF and tissue reflectance (blood volume) under HBO. We also examined, for the first time, the relationship between microcirculatory blood flow, intracellular mitochondrial NADH and microcirculatory HbO_2 under various O_2 pressures (from 1 ATA to 6 ATA). Such data could significantly contribute to the understanding of brain energy metabolism in normobaric and hyperbaric oxygenation.

2. Results

Anoxia (100% N_2 inhalation for about 20 seconds) was induced in order to find the maximal reduced NADH and minimal HbO_2 levels. The effect of anoxia on the measured parameters is shown in Fig. 1. ANOVA (repeated measures) showed significant changes in CBF, reflectance, NADH and HbO_2 ($F(9,26) = 8.408$, $p = 0.001$; $F(9,26) = 39.432$, $p < 0.001$; $F(9,26) = 51.623$, $p < 0.001$; $F(9,26) = 21.110$, $p < 0.001$, respectively). Additionally, Bonferroni *post hoc* tests showed that immediately after 100% N_2 inhalation, CBF, reflectance and HbO_2 decreased significantly ($p < 0.01$, $p < 0.001$ and $p < 0.001$ respectively), whereas mitochondrial NADH increased (about 35%) significantly

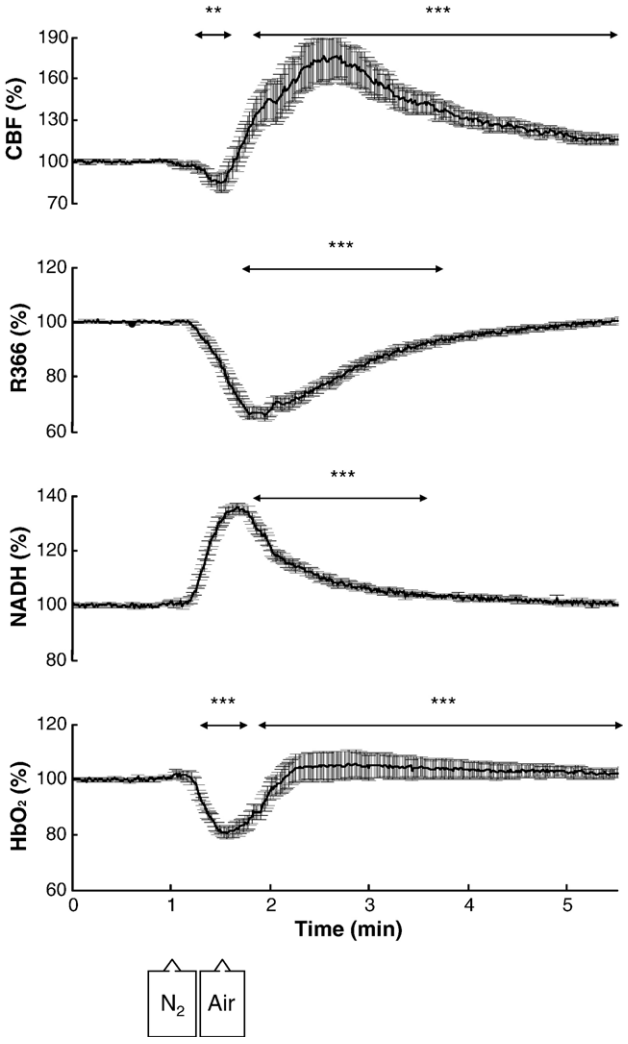

Fig. 1 – The effect of anoxia (100% N_2) on cerebral blood flow (CBF), reflectance (R366), mitochondrial NADH redox state (NADH) and hemoglobin oxygen saturation (HbO_2). Values are shown as mean percent values ± SE. $^{**}p<0.01$, $^{***}p<0.001$ range of time showing significant differences found by ANOVA repeated measures.

($p<0.001$). The maximum decrease in HbO_2 and augmentation in NADH during the anoxia period were calculated from all experimental groups and were −25.14 ± 1.39% and 42.66 ± 1.12%, respectively. Returning to spontaneous air breathing caused a significant ($p<0.001$) increase (about 75%) in CBF that showed a trend of returning to baseline levels about 1 min after starting spontaneous air breathing. Reflectance, NADH redox state and HbO_2 also showed a trend of returning to normal levels during spontaneous air breathing.

One group of rats was tested to show the effect of HH at various "non-toxic" pressure levels (1.5, 2, 2.5, 3 and 3.5 ATA) on the measured parameters (Fig. 2). Since the range of therapeutic HBO pressure levels confirmed by the Undersea and Hyperbaric Medical Society (UHMS) for various pathological conditions was between 2 and 3 ATA (HBO approved indications; Feldmeier, 2003; Gill and Bell, 2004), we decided to test the effect of these pressures, compared to the control group at 0.2 ATA, on the measured parameters. The experiments started from normal pressure (0.2 ATA) for 15 min. Then, pressure was elevated gradually starting from (NH) at 1 ATA and continued to hyperbaric pressure elevating by increments of 0.5 ATA from 1.5 to 3.5 ATA, after washing the chamber with 100% O_2. Each step of pressure continued for 15 min, HbO_2 increased significantly ($F(6,9)=20.560$, $p<0.001$) and NADH redox state decreased significantly ($F(6,9)=20.890$, $p<0.001$). The 366-nm reflectance augmented significantly ($F(6,9)=11.242$, $p=0.001$) showing a decrease in blood volume, whereas CBF did not demonstrate significant changes. In addition, Bonferroni *post hoc* tests showed significant

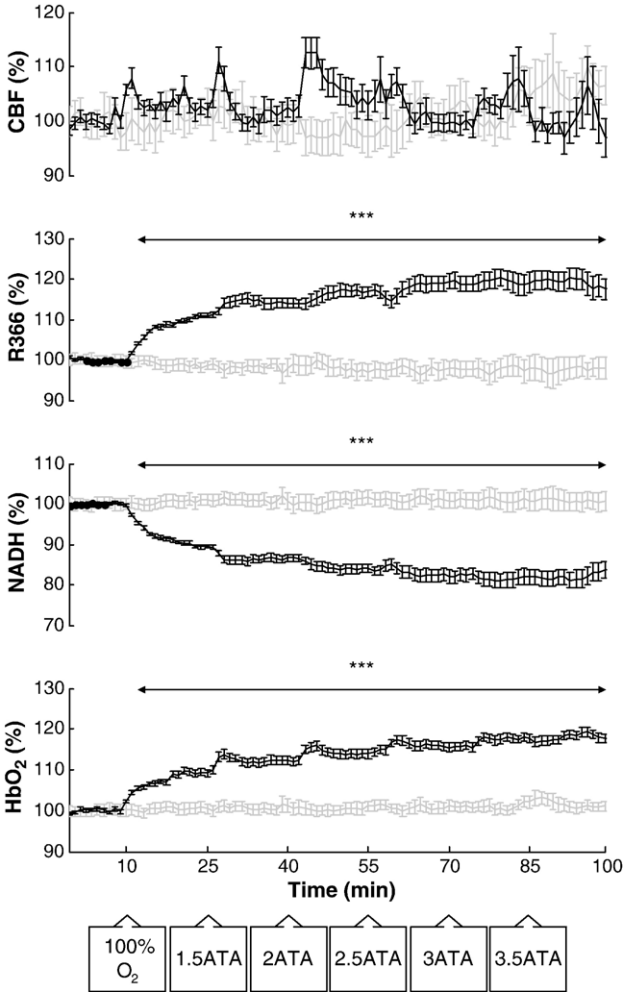

Fig. 2 – The effect of normobaric hyperoxia (100% O_2) and various levels of hyperbaric hyperoxia (1.5 ATA, 2.0 ATA, 2.5 ATA, 3 ATA and 3.5 ATA) on cerebral blood flow (CBF), reflectance (R366), mitochondrial NADH redox state and hemoglobin oxygen saturation (HbO_2). The data are presented as mean percent values ± SE. Black points—experimental group; gray points—control group. Repeated measures ANOVA and Bonferroni *post hoc* test showed significant differences between control and experimental group. $^{***}p<0.001$ range of specific time points (20, 35, 50, 65, 80 and 95 min) presenting significant differences calculated by the Student *t*-test between the control and the experimental groups.

differences (between $p<0.05$ to $p<0.001$) between the control values at 0.2 ATA (at 5 min of recording) and almost all other time points (20, 35, 50, 65, 80 and 95 min), where the parameters reached steady state for each level of pressure induced. These results show that the gradual increases in hyperbaric pressures were significantly different compared to normoxia (0.2 ATA) in reflectance, NADH redox state and HbO_2. Significant differences ($p<0.001$) were also found between the control and experimental groups at the same 6 time points (from 20 to 95 min) showing a significant effect of 100% O_2 and the various levels of hyperbaric pressure.

The effects of HH (100% O_2) at various pressure levels (1.75, 2.5, 4.5 and 6 ATA) on CBF, reflectance, mitochondrial NADH redox state and HbO_2 during the initial 60 min of experiment are shown in Fig. 3. No significant changes were observed in CBF. The reflected light increased significantly as a result of

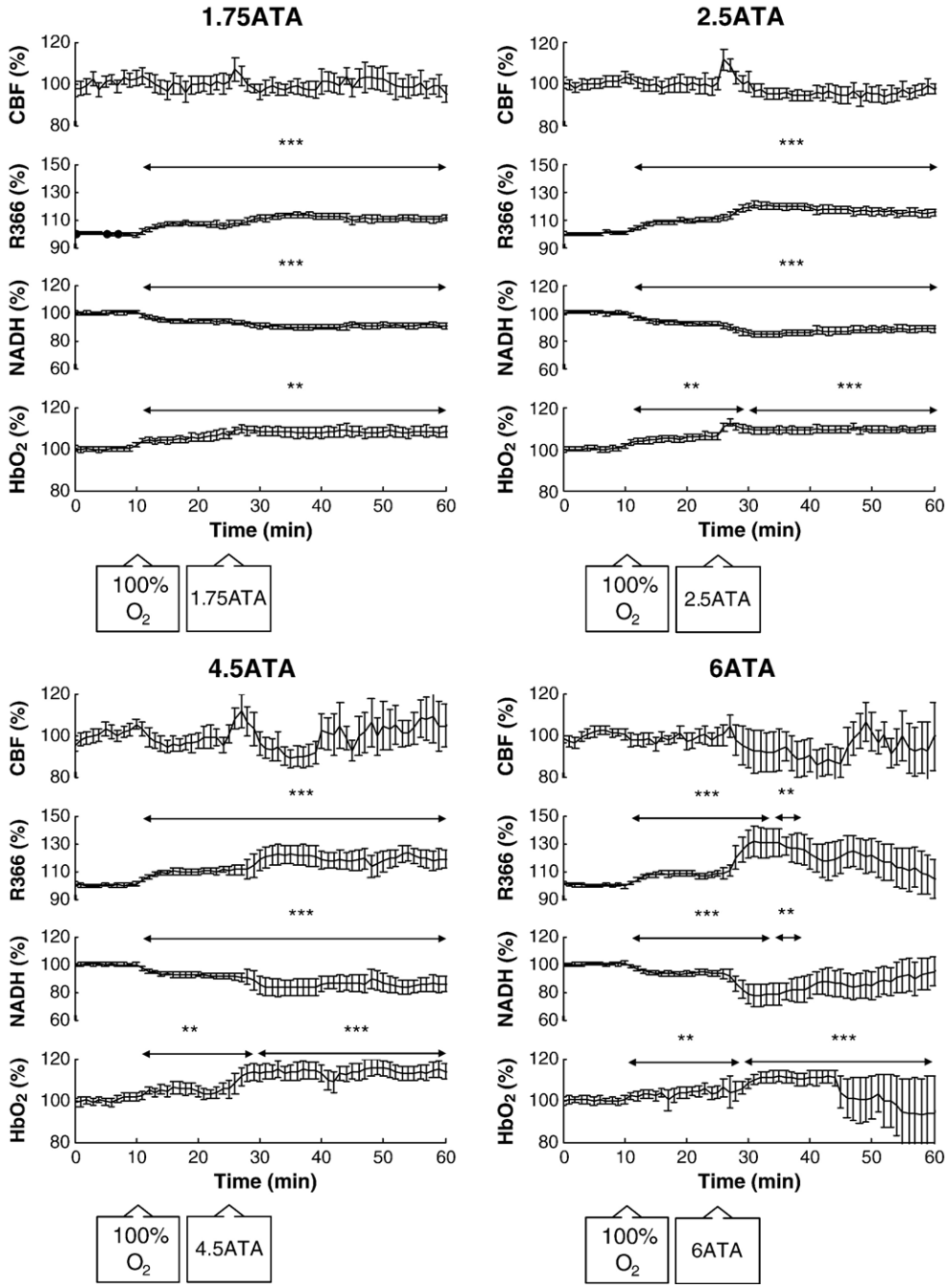

Fig. 3 – The effect of normobaric hyperoxia (100% O_2) and various levels of hyperbaric hyperoxia (1.75 ATA, 2.5 ATA, 4.5 ATA and 6 ATA) on changes, with respect to control (normoxia), in cerebral blood flow (CBF), reflectance (R366), mitochondrial NADH redox state and hemoglobin oxygen saturation (HbO_2) during the first 60 min of the experiment. Values are shown as mean percent values ± SE. Repeated ANOVA and Bonferroni *post hoc* test showed significant differences between control and experimental groups. $^{**}p<0.01$ or $^{***}p<0.001$ range of specific time points (10, 20, 30, 40 and 60 min) presenting significant differences calculated by the Student *t*-test between the control (data not shown) and the experimental groups.

100% O_2 inhalation (NH) and under each of the four HH pressure levels (1.75, 2.5, 4.5 and 6 ATA) induced ($F(5,8)=5.714$, $p=0.015$; $F(5,8)=15.739$, $p=0.001$; $F(5,8)=7.758$, $p=0.006$; $F(5,8)=3.933$, $p=0.043$, respectively). Furthermore, in the 6 ATA group, reflectance decreased after 45 min of the experiment. NADH decreased (became oxidized) significantly during NH and also during HH at each of the four pressures ($F(5,8)=6.772$, $p=0.009$; $F(5,8)=14.361$, $p=0.001$; $F(5,8)=7.801$, $p=0.006$; $F(5,8)=7.749$, $p=0.006$, respectively), almost inversely proportional to the changes in reflectance. HbO_2 augmented significantly ($F(5,8)=33.263$, $p<0.001$; $F(5,8)=17.211$, $p<0.001$; $F(5,8)=2.908$, $p=0.05$; $F(5,8)=7.047$, $p=0.008$, respectively) during NH and HH. In addition, Student *t*-test showed that hemoglobin oxygenation was seen immediately after 100% O_2 inhalation ($p<0.01$) and after each one of the pressures induced a further hemoglobin oxygenation was found ($p<0.01$ or $p<0.001$). This increase reached a steady-state level that persisted until the end of the experiment (except at 6 ATA).

To analyze the results in detail, we focused on two parameters, namely, NADH and HbO_2. The minimal NADH levels and the maximal HbO_2 values were selected during the three phases: normoxia (0.2 ATA), NH and HH, in each experiment. Hyperbaric treatment was applied to four groups (1.75, 2.5, 4.5 and 6 ATA, respectively). The mean values for all groups, during each phase, as well as the standard error of means, are shown in Table 1. A strong influence of pressure was found on NADH and HbO_2 (Table 1). We found a significant effect of oxygen in the transition between the phases of normoxia, NH and HH. Paired *t*-test showed that in the transition from normoxia to NH, NADH decreased (became oxidized) ($p<0.001$) and at the same time HbO_2 increased ($p<0.01$). Furthermore, NADH showed an additional decrease (oxidation) in the transition from NH to HH ($p<0.001$) and a further hemoglobin oxygenation was found ($p<0.01$).

Two approximation models were found for NADH vs. hyperbaric pressure (HP) and HbO_2 vs. HP (Fig. 4A) with the help of curve fitting procedure of SPSS program. In both cases, the mathematical model showed an asymptotic curve of the formula HP / (HP + $HP_{(50)}$). The constant value for NADH was $HP_{(50)}=-0.155\pm0.03\%$ (confidence interval: −0.086 to −0.225), and for HbO_2 it was $HP_{(50)}=0.266\pm0.05\%$ (confidence interval: 0.163 to 0.368). The approximation line in the figure gives estimations for 0.2 ATA and 1.75 ATA interval of steep changes where no additional experimental points (pressures) exist. The plateau of the asymptotic curve can explain why at low pressures (up to about 2.5 ATA) the tissue becomes oxidized faster than under high pressures (4.5 and 6 ATA).

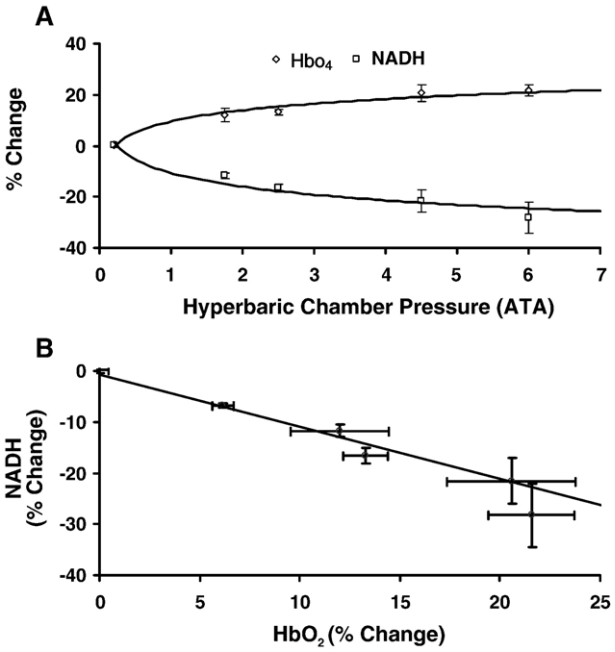

Fig. 4 – (A) An asymptotic curve fitting of a mathematical approximation model for NADH vs. hyperbaric pressure (HP) and HBO_2 vs. HP. The constant for NADH is $HP_{(50)}=-0.155\pm0.03\%$ (confidence interval: −0.086 to −0.225), and for HbO_2 it is $HP_{(50)}=0.266\pm0.05\%$ (confidence interval: 0.163 to 0.368). (B) Linear and logarithmic curve fitting of mathematical approximation models for NADH vs. HbO_2. Linear: NADH = −1.018 (HbO_2), $R^2=0.8605$, $p<0.001$; logarithmic: NADH = −53.862 ln (HbO_2) + 149.38, $R^2=0.6236$, $p=0.002$.

Table 1 – The effect of transition from normoxia (0.2 ATA) to normobaric hyperoxia (NH), 100% O_2 and from NH to various levels of hyperbaric hyperoxia (HH) on peak values of NADH and HbO_2, evaluated in the experimental groups illustrated in Fig. 3

Group (ATA)	Normobaric hyperoxia		Hyperbaric hyperoxia	
	Mean ± SE		Mean ± SE	
	NADH	HBO_2	NADH	HBO_2
1.75	94.2 ± 0.6***	105.9 ± 1.4**	88.2 ± 1.2***	112.0 ± 2.4**
2.5	92.9 ± 0.7***	105.8 ± 1.0**	83.4 ± 1.5***	113.3 ± 1.1**
4.5	92.3 ± 1.1***	106.3 ± 1.1**	78.4 ± 4.4***	120.6 ± 3.2**
6.0	93.5 ± 1.1***	106.6 ± 1.0**	71.8 ± 6.2***	121.6 ± 2.1**

Data presented in mean percents values ± SE. NH values were compared to control values in normoxia (100%) and HH values were compared to NH. **$p<0.01$ ***$p<0.001$.

We also performed the approximation curve fitting for the relation NADH vs. HbO_2. Two mathematical models were built: a linear and a logarithmic (Fig. 4B). For the logarithmic regression approximation, we did not use nitrogen inhalation values which were normalized to 0%. We omitted these values for this curve since we did not examine the data for 0–50% hemoglobin saturation and NADH oxygenation change. The linear and logarithmic regression approximations between hemoglobin oxygenation and mitochondrial NADH oxygenation under different levels of hyperbaric pressures are linear: NADH = −1.018 (HbO_2), $R^2=0.8605$, $p<0.001$; and logarithmic: NADH = −53.862 ln (HbO_2) + 149.38, $R^2=0.6236$, $p=0.002$.

A significant correlation (Pearson) was found between reflectance 366 nm vs. reflectance 585 nm during N_2 inhalation ($r=0.917$, $p<0.001$, $n=60$). The correlation coefficient was calculated using averaged data from 35 rats. Furthermore, a significant correlation ($r=0.965$, $p<0.001$, $n=7$) was also found between the steady state values of the same variables during

normoxia, NH and various levels of HH (1.5, 2, 2.5, 3 and 3.5 ATA). This correlation coefficient was calculated using averaged data from 7 rats.

3. Discussion

This study examines the relationship between microcirculatory blood flow, tissue reflectance, hemoglobin oxygenation and intracellular mitochondrial NADH redox state under normoxia (21% O_2), normobaric hyperoxia (100% O_2) and variable levels of hyperbaric hyperoxia. This combination of monitored parameters has not been measured before, and the availability of such data may contribute significantly to the understanding of brain energy metabolism *in vivo* at various levels of oxygen supply above normoxia.

Consequently, important information was to be gained on the relationship between brain tissue O_2 delivery, O_2 consumption and utilization (mitochondrial NADH redox state). In all experiments the rats were monitored in room air while located inside the hyperbaric chamber. Under these conditions the CO_2 is washed out from the chamber by a continuous leak of the O_2 from the chamber. We showed that when CO_2 was added to the chamber (1–5% CO_2) the typical responses, in NADH oxidation, to hyperbaric oxygenation were recorded (Mayevsky, 1978b). The pentobarbital was one of the components of the anesthetic mixture used. Therefore, the amount of pentobarbital given to each rat was relatively small as compared to the dose given when the pentobarbital is the sole anesthetic drug used. In addition, in order to avoid the residual effects of the pentobarbital, the pressure elevation was started 4 h after the initial injection of the mixture of anesthetic given and the rat was in the awake state as was observed in the ECoG signal.

It is important to note that the multiparametric monitoring system described here provides several wavelengths ranging from 366 nm, 577, 585 and 632 nm (laser Doppler). All signals are measured from a depth of up to 1 mm depending on the specific wavelength. In the rat, the depth of the exposed parietal cortex is close to 2 mm, and therefore all the parameters are measured from a similar cortical tissue that is affected similarly by the hyperbaric oxygenation.

In this study as well as in previous investigations (Mayevsky, 1975; Mayevsky et al., 1974, 1989, 1980; Mayevsky and Rogatsky, 2007; Mayevsky and Shaya, 1980; Yoles et al., 2000), we showed that the reflectance changes occurring immediately after increasing oxygen levels are due to changes in the hemodynamic responses tending towards vasoconstriction (a decrease in blood volume). This vasoconstrictive effect was shown by a reduction trend in CBF and a significant increase in reflectance, indicating a significant decrease of blood volume in the awake rat brain under various oxygen levels. The decrease in blood volume was found in our previous studies (Mayevsky et al., 1974, 1989; Yoles et al., 2000). Parallel to the decrease in blood volume, we observed an oxidation of brain NADH (Figs. 2 and 3), suggesting that NADH is not fully oxidized in the normoxic brain *in vivo* and can be oxidized even further under hyperbaric hyperoxic conditions.

In the current study we used hyperbaric oxygenation as a tool to study the HbO_2 levels in microcirculation during a wide range of pressures (including the toxic levels, 4.5 ATA and 6 ATA; data not shown).

The effect of hyperbaric oxygenation on CBF was found to be highly dependent on the CBF monitoring method. Studies using laser Doppler flowmetry (Chavko et al., 1998; Sato et al., 2001; Thom et al., 2002; Zhang et al., 1995) revealed no systematic changes or only a small decrease or increase under exposure to 1–4 ATA. At pressures above 4 ATA, in the first few minutes, the CBF decreased and returned to the base line within a variable period. Several minutes before convolutions onset, the CBF rose significantly. Studies using the hydrogen clearance method to measure CBF (Demchenko et al., 2000, 2001, 2005; Moskvin et al., 2003; Zhilyaev et al., 2003), under low pressures (1–4 ATA), showed a significant decrease in CBF which remained stable until the end of the experiments. Under 4–5 ATA, within the first few minutes, CBF decreased significantly and afterwards suddenly rose significantly. At 6 ATA, CBF increased immediately. Using radiolabeled microspheres (Bergo and Tyssebotn, 1992), a decrease in CBF was seen at 1–5 ATA. A decrease in CBF was also found using the transcranial Doppler in humans (Omae et al., 1998) under 2 ATA. A significant increase in CBF, before convulsions onset, was also observed when using thermoflow probes (Bean et al., 1971).

In pathological conditions such as ischemia or hypoxia, pO_2 measurements reliably reflect cerebral oxygen delivery and consumption (Scheufler et al., 2002). A clear linear relationship has been found between the O_2 partial pressure in the brain tissue and the hyperbaric chamber O_2 pressure (van Hulst et al., 2003). Mean oxygen tension in the brain tissue rises consistently with an increase in the hyperbaric chamber pressure. However, the distribution of this oxygen in the tissue microcirculation remains unclear. Questions arise at to how much of this oxygen is actually bound to hemoglobin; how much oxygen is dissolved in the plasma of the circulating blood; and how the relationship between the two O_2 carrying mechanisms is affected by hyperbaric oxygenation.

We present, for the first time as far as we know, an approximation for the amount of oxygen actually bound to hemoglobin in the hyperoxic brain tissue. We found that the hemoglobin saturation in the brain tissue increases by an asymptotic curve as the chamber pressure rises, approximating a plateau at about 2.5 ATA (Fig. 4A). This observation is supported by others (Araki et al., 1988). This may imply that tissue HbO_2 is in close proximity to the maximal value at 2.5 ATA, and pressures above 2.5 ATA do not significantly alter the tissue hemoglobin saturation. The significant increase in HbO_2, resulting from increased oxygen partial pressure, evidences that brain microcirculatory hemoglobin was not fully oxygenated under normal conditions and it reached a maximal saturation at 2.5 ATA (Figs. 2, 3 and 4A), when all O_2 supply to the cells was provided by the O_2 dissolved in the plasma. Elevation of the pressure above 2.5 ATA did not further oxygenate the hemoglobin (Figs. 2, 3 and 4A), and no changes were found in HbO_2 levels.

We assume that the amount of hemoglobin in the blood is constant (during the experiment) and that the number of oxygen binding sites in each hemoglobin molecule is four.

Presumably, at 2.5 ATA, almost all hemoglobin binding sites became occupied by oxygen, hence the full tissue hemoglobin saturation at 2.5 ATA. At the maximal hemoglobin saturation, hemoglobin affinity for oxygen decreases (Mathews et al., 1999), resulting in a greater probability of oxygen release. Since the concentration of dissolved oxygen in the tissue is high, the oxygen molecules released from hemoglobin will be replaced by others from the plasma. This can explain the plateau found in tissue HbO_2.

The effect on the energy balance induced by HBO was shown by mitochondrial NADH status (Figs. 2, 3 and 4A). A similar oxygenation effect was found in our previous studies (Mayevsky, 1975; Mayevsky et al., 1974, 1989, 1980; Mayevsky and Shaya, 1980; Yoles et al., 2000). Following pressure exertion, we observed a significant oxidation of mitochondrial NADH. The NADH oxidation curve behaves almost in parallel to HbO_2, the flattening of the asymptotic curve with the chamber pressure rise occurring at about 2.5 ATA. In our previous work, we tested various pressures ranging 3–11 ATA (Mayevsky and Shaya, 1980) and found no additional NADH oxidation under all those pressures. Even though NADH clearly oxidizes and reaches a plateau, we cannot determine whether the oxygen oxidizes all of the NADH at the beginning with no subsequent consumption, or that oxygen also stimulates tissue metabolism. It must be emphasized that NADH is normalized with maximum O_2 oxygenation, but it is possible that other factors, such as glucose, can lead to an even further oxygenation.

The behavior of hemoglobin oxygenation and mitochondrial NADH oxidation under different hyperbaric pressures yielded two possible curves: logarithmic and linear regression approximation curves between these two parameters (Fig. 4B). Thus, increasing hemoglobin saturation causes oxidation of mitochondrial NADH. Both curves are plausible, but since the first 50% of the linear curve is estimated, we prefer the logarithmic approximation. This estimation under hypoxic conditions will be further tested in our future studies.

Mayevsky and co-workers (Mayevsky, 1975; Mayevsky et al., 1974) showed that the reflectance at 366 nm is inversely correlated to the expected changes in the tissue blood volume. The significant Pearson correlation coefficients found between reflectance at 366 nm and reflectance at 585 nm shows that the 585-nm reflectance also estimates tissue blood volume and therefore can also be used as a hemodynamic parameter.

In conclusion, the present work shows that even though oxygen tension in the brain tissue rises consistently with the increase in pressure in the hyperbaric chamber, hemoglobin oxygenation increases up to 2.5 ATA. At higher pressures, hemoglobin oxygenation levels off. This shows that at 2.5 ATA, the maximum saturation of hemoglobin in the tissue is achieved. Therefore, higher hyperbaric pressures produce no further hemoglobin oxygenation and mitochondrial NADH oxidation. These results may imply that, at 2.5 ATA, hemoglobin is 100% saturated and the tissue cells become 100% oxygenated, so that additional O_2 local partial pressure will not affect the mitochondrial redox state.

This information is vital because such pressures (around 2.5–3 ATA) are standard clinically applied pressures used to treat most of the pathophysiological problems defined by the Undersea and Hyperbaric Medical Society (UHMS) (HBO approved indications; Feldmeier, 2003; Gill and Bell, 2004). When considering clinical applications of HBO therapy, the potential benefit must be balanced against the potential toxicity. In other words, our work shows that most of the oxidation of NADH and the oxygenation of HbO_2 take place in the proximity of 2.5–3 ATA. Additional oxygen pressure caused brain oxygen toxicity within a short variable period of time after the pressure elevation (HBO approved indications; Chavko et al., 1998; Mayevsky, 1975, 1983; Mayevsky et al., 1974, 1980, 1986; Mayevsky and Shaya, 1980).

4. Experimental procedures

In order to assess the hemodynamic and metabolic functions of the cerebral cortex, we used the Tissue Vitality Monitoring System (TVMS) that includes two devices: a time-sharing fluorometer-reflectometer (TSFR) for mitochondrial NADH redox state and microcirculatory hemoglobin oxygen saturation HbO_2 measurement (Rampil et al., 1992) combined with a laser Doppler flowmeter (LDF) for CBF monitoring (Fig. 5). The connection between the brain and the TVMS was done by a flexible light guide inserted through the wall of the hyperbaric chamber. The fiber optic probe includes fibers that were connected to the two instruments as shown in the enlargement of the bundle tip. The measurement of HbO_2, reflectance at 366 nm and NADH were performed by the same excitation and emission fibers. The CBF was measured by 3 optical fibers located in the center of the time sharing bundle of fibers. This system includes a rotating wheel with 8 specific filters at the appropriate wavelengths (366 nm, 450 nm for NADH and 585 nm, 577 nm for oxyhemoglobin measurement) to 4 filters for excitation light and 4 filters for emitted light. The wheel rotates at about 2400 rpm, which is a very high speed with respect to the kinetics of physiological changes; thus, NADH and oxyhemoglobin are simultaneously monitored (Mayevsky and Rogatsky, 2007; Rampil et al., 1992).

4.1. *Microcirculatory blood flow*

The optical fibers of the laser Doppler flowmeter were used for monitoring tissue blood flow (TBF). The LDF measures relative changes (in the 0–100% range), which are significantly correlated to the absolute values of TBF measured by other quantitative methods: H_2 clearance (Dirnagl et al., 1989), (^{14}C) iodoantipyrine (Haberl et al., 1989) and radioactive microspheres (Kirkeby et al., 1995).

4.2. *NADH redox state fluorometer/reflectometer*

The principle of NADH monitoring from the tissue surface is that excitation light (366 nm) is passed from the fluorometer to the tissue via a bundle of quartz optical fibers. When mitochondrial NADH is illuminated by the excitation light, fluorescent light is emitted at 420–480 nm. The emitted light, together with the reflected light at the excitation wavelength (366 nm), is transferred to the fluorometer via another bundle of optical fibers. It is known that reflectance at 366 nm is

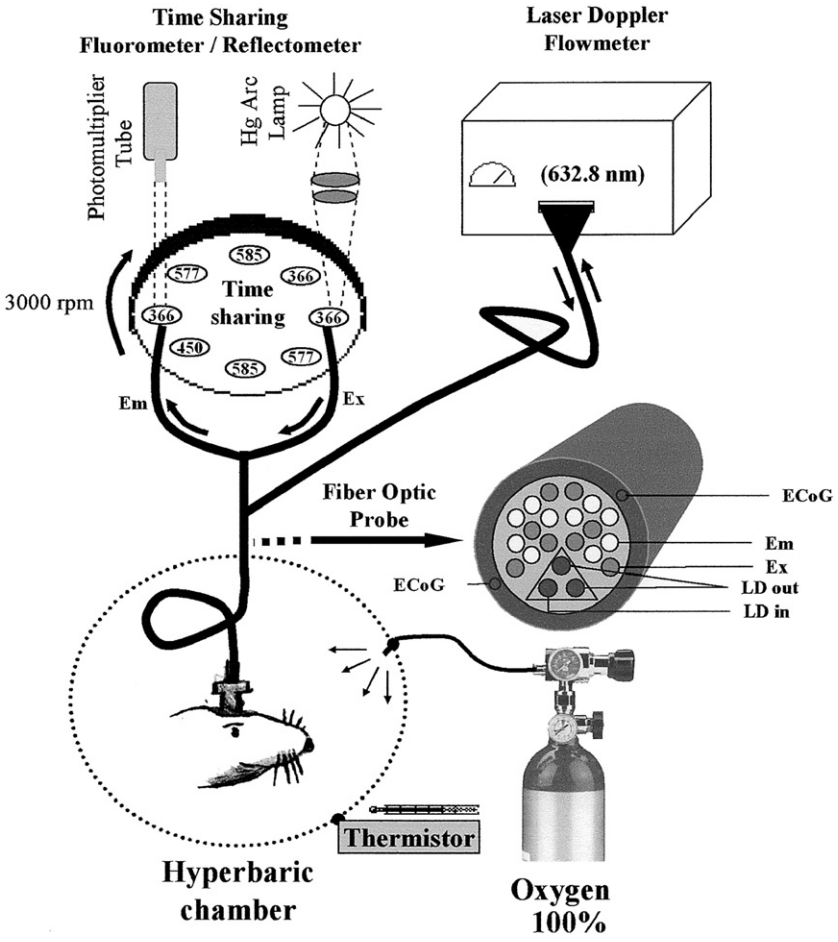

Fig. 5 – Schematic representation of the experimental setup showing the time-sharing fluorometer reflectometer, laser Doppler flowmeter and electrodes for electrocorticography (ECoG). Ex and Em—excitation and emission fibers for NADH and HbO_2 monitoring, respectively; LD in and LD out—optical fibers for blood flow monitoring. The numbers in the spinning disk refer to the wavelength filters. The rat brain is connected to monitoring system via a flexible fiber optic probe penetrating the wall of the chamber.

inversely correlated to changes in the tissue blood volume (Dora, 1985; Mayevsky, 1975; Mayevsky et al., 1992, 1974; Mayevsky and Rogatsky, 2007; Rampil et al., 1992); therefore, the reflectance can be used as a hemodynamic parameter. The NADH fluorescence trace was corrected for hemodynamic artifacts by subtracting the reflectance signal (at 366 nm) from the fluorescence (450 nm) signal (Mayevsky et al., 1992; Mayevsky and Chance, 1982). A detailed discussion about this issue can be found in our review articles (Mayevsky, 1984; Mayevsky and Rogatsky, 2007). The changes in the fluorescence and reflectance signals are calculated relative to the calibrated signals under normoxic conditions (100% NADH) (Mayevsky, 1978a, 1984; Mayevsky et al., 2002, 2004, 1998). The correction of the NADH fluorescence signal for changes in tissue absorption and scattering was initiated in the late 1960s and 70s by various investigators (Chance and Legallias, 1963; Jobsis et al., 1971; Jobsis and Stainsby, 1968). Most of the published material in this field of *in vivo* NADH monitoring of tissues used the subtraction of the reflectance signal (366 nm) from that of the NADH fluorescence signal measured to provide the corrected fluorescence signal (Dora et al., 1984; Harbig et al., 1976). Few attempts were done in order to improve the correction technique but no significant improvement was found. In addition, the various correction techniques were listed and discussed in details in the paper published by Ince et al. (1992). Most of the published material was based on 1:1 ratio, when subtracting the 366-nm reflectance from the 450-nm fluorescence signal. We have found that subtracting the reflectance from the fluorescence or dividing the two parameters provides similar net NADH changes. Kramer and Pearlstein (1979) published a very short report in Science in 1979 and suggested using other wavelengths in the fluorometer in order to better monitor the NADH but neither the same group nor other groups were able to use this approach and no further publications have been found. Also, Coremans et al. (1997) discussed the correction issue and summarized "Although Kramer and Pearstein argued that algebraic subtraction of the reflectance from the NADH fluorescence is inappropriate and a ratio technique should be used instead, studies based on fluctuation in the intensity of UV reflectance of a small tissue area yielded inconsistent results" (Kramer and Pearlstein, 1979). Very

recently, Bradley and Thorniley (2006) reviewed the available techniques for correction of fluorescence signals. They concluded, "even though research has been conducted into correction techniques for over thirty years, the development of a successful and practical correction technique remains a considerable challenge".

4.3. *Hemoglobin oxygenation reflectometer*

Oxyhemoglobin measurement is based on the differences in the absorption properties of hemoglobin in its oxygenated vs. deoxygenated states. The tissue is illuminated at two wavelengths: 585 nm and 577 nm. At 585 nm, oxyhemoglobin and deoxyhemoglobin have the same absorption characteristics (the isosbestic point); thus, the light emitted from the tissue reflects changes in tissue blood volume. At 577 nm, oxyhemoglobin has a higher absorption ability (lower reflectance) than deoxyhemoglobin; thus, at this wavelength, the emitted light intensities are affected by the oxygenation levels as well as by the changes in blood volume at the measurement site. Therefore, subtracting changes in 585 nm reflectance from those at 577 nm provides a parameter correlated to net changes in blood oxygenation (Deutsch et al., 2004; Rampil et al., 1992).

4.4. *Animal preparation*

All experiments were performed in accordance to the Guidelines of the Animal Care Committee of Bar-Ilan University. Male Wistar rats ($n=42$) weighing 220–320 g, were used in all experiments. The animals were anesthetized by Equithesin (each ml contains: pentobarbital 9.72 mg, chloral hydrate 42.51 mg, magnesium sulfate 21.25 mg, propylene glycol 44.34% w/v, alcohol 11.5% and water) using intraperitoneal (IP) injection with a minimal amount of anesthetic (0.3 mg/100 g body weight). The head was fixed in a head holder, the skull exposed and a 6-mm-diameter hole drilled in the left parietal bone. A black Delrin light-guide holder (cannula) was combined with silver wires for measuring a bipolar electrocorticogram (ECoG). The cannula was placed epidurally to provide a constant contact between the light guide (inserted into the cannula) and the brain (Mayevsky, 1984). Four stainless steel screws were implanted: three in the right hemisphere and one in the left. The cannula and the screws were cemented to the skull using dental acrylic cement. This procedure fixated the cannula to the brain and prevented movements of the probe on the brain avoiding artifact recordings in the measured parameters, in the awake animals. The dental acrylic cement also prevented leakage of oxygen around the fiber bundle during HH. A thermistor (Yellow Springs Instruments Co. Inc., type 402) was inserted into the rectum for continuous monitoring of body temperature that was kept constant at 36–37 °C. The animal was placed in a Plexiglas cage, fitted to the rat's size and was allowed to recover for about 30 min. The normal response to anoxia was checked by the NADH increase and HbO_2 decrease. Thereafter, the rat (in the Plexiglas cage) was placed in a 150-l hyperbaric chamber, containing a special cage heated by water (Bethlehem Corp. FM-21-A). Since the effects of HBO were performed in the awake state, we did not measure systemic blood pressure which may add another complication to the model or to the rat located in a confined environment.

4.5. *Experimental procedures*

Four hours after anesthesia, the rats were observed in a fully awake state and the experiment started. The chamber was flushed with 100% oxygen, normobaric hyperoxia (NH) for 15 min at atmospheric pressure (cleaning phase) in order to prevent acid base disturbances and physiological CO_2 elevation in blood. Then, the oxygen pressure was elevated at a rate of 1 atmosphere per minutes to a maximum pressure of the hyperbaric hyperoxia (HH). The respective maximum pressures were applied to four groups of animals. Rats of group 1 ($n=7$) were subjected to a maximum pressure of 1.75 ATA for 90 min. Group 2 ($n=7$) was exposed to a maximum pressure of 2.5 ATA for 90 min. Ten minutes after decompression, the animals of those two groups were exposed to pure N_2 inhalation until death. During the decompression of the hyperbaric chamber, the pressure dropped immediately to reach room pressure. Group 3 ($n=7$) was subjected to a pressure of 4.5 ATA until the appearance of the first tonic-clonic convulsions, and then decompression was performed. Ten minutes later the animals were exposed to pure N_2 inhalation until death. Group 4 ($n=7$) was pressurized to a maximum of 6 ATA. The animals were left in the chamber until their death that occurred approximately 2 h after compression induction. Another group of rats ($n=7$) was exposed to a rise in gradient pressure (0.5 ATA), starting from normal pressure (0.2 ATA), normobaric hyperoxia (100% O_2) at 1 ATA (NH) and continuing by elevating the pressure from 1.5–3.5 ATA every 15 min. Decompression was then performed and 15 min later under new steady state conditions, the rat was exposed to N_2 inhalation until death. An additional group of rats ($n=7$), a control group, underwent the same preparation and procedures, and the rats were monitored at 0.2 ATA of air in the chamber for 2.5 h, without pressure, and were exposed to pure N_2 inhalation to end the measurement period.

4.6. *Data collection, processing and statistical analysis*

Since neither optical signal is calibrated in absolute units, we used the standard approach of calibration and presentation. After connecting the operated brain to the monitoring system, all signals were set electronically to read 100% of the normoxic brain. Although this number does not represent the real level of NADH or HbO_2, we defined it as 100% and measured the percent change of the signal. In this work, for the first time, we determined both levels of hemoglobin saturation in the brain and were able to normalize the results, 0% representing minimal oxygenation (nitrogen inhalation) and 100% for maximal oxygenation (6 ATA O_2).

Data were collected and stored in computer files, using LabView A/D hardware and software (National Instruments Inc., USA). Simultaneously, electrocorticogram (ECoG) and electrocardiogram (ECG) were recorded by a polygraph.

The effects of normoxia, normobaric hyperoxia and hyperbaric hyperoxia within the control and experimental groups were tested by one-way repeated measures analysis of variance (ANOVA-RM) with Bonferroni *post hoc* test to evaluate

the specific time point significance. Additionally, the difference between the control and the experimental groups at specific time points was also tested by separate Student *t*-tests or paired *t*-tests between two specific time points in the experimental group.

Mathematical approximation models (HbO_2 vs. Pressure (ATA) and NADH vs. Pressure (ATA)) were calculated via ANOVA repeated measures and via non-linear regression analysis with an asymptotic curve as a possible approximation. For the curve fitting of HbO_2 vs. NADH, we used both non-linear (logarithmic) and linear approximations. Pearson correlation analysis was performed to elucidate the relation between reflectance 366 nm vs. reflectance 585 nm. *P* values less then 0.05 were regarded as significant. All analyses were performed with SPSS statistical program (version 13, SPSS Inc., Chicago, IL, USA).

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REFERENCES

Araki, R., Nashimoto, I., Takano, T., 1988. The effect of hyperbaric oxygen on cerebral hemoglobin oxygenation and dissociation rate of carboxyhemoglobin in anesthetized rats: spectroscopic approach. Adv. Exp. Med. Biol. 222, 375–381.

Bean, J.W., Lignell, J., Coulson, J., 1971. Regional cerebral blood flow, O_2, and EEG in exposures to O_2 at high pressure. J. Appl. Physiol. 31, 235–242.

Bergo, G.W., Tyssebotn, I., 1992. Cerebral blood flow distribution during exposure to 5 bar oxygen in awake rats. Undersea Biomed. Res. 19, 339–354.

Bradley, R.S., Thorniley, M.S., 2006. A review of attenuation correction techniques for tissue fluorescence. J. R. Soc. Interface 3, 1–13.

Chance, B., Legallias, V., 1963. A spectrofluorometer for recording of intracellular oxidation–reduction states. IEEE Trans. Biomed. Eng. BME-10, 40–47.

Chance, B., Williams, G.R., 1955. Respiratory enzymes in oxidative phosphorylation. III. The steady state. J. Biol. Chem. 217, 409–427.

Chance, B., Oshino, N., Sugano, T., Mayevsky, A., 1973. Basic principles of tissue oxygen determination from mitochondrial signals. In: Bicher, H.I., Bruley, D.F. (Eds.), Oxygen Transport to Tissue. Instrumentation, Methods, and Physiology. Plenum Publishing Corporation, New York, pp. 277–292.

Chavko, M., Braisted, J.C., Outsa, N.J., Harabin, A.L., 1998. Role of cerebral blood flow in seizures from hyperbaric oxygen exposure. Brain Res. 791, 75–82.

Coremans, J.M., Ince, C., Bruining, H.A., Puppels, G.J., 1997. (Semi-) quantitative analysis of reduced nicotinamide adenine dinucleotide fluorescence images of blood-perfused rat heart. Biophys. J. 72, 1849–1860.

Demchenko, I.T., Boso, A.E., O'Neill, T.J., Bennett, P.B., Piantadosi, C.A., 2000. Nitric oxide and cerebral blood flow responses to hyperbaric oxygen. J. Appl. Physiol. 88, 1381–1389.

Demchenko, I.T., Boso, A.E., Whorton, A.R., Piantadosi, C.A., 2001. Nitric oxide production is enhanced in rat brain before oxygen-induced convulsions. Brain Res. 917, 253–261.

Demchenko, I.T., Luchakov, Y.I., Moskvin, A.N., Gutsaeva, D.R., Allen, B.W., Thalmann, E.D., Piantadosi, C.A., 2005. Cerebral blood flow and brain oxygenation in rats breathing oxygen under pressure. J. Cereb. Blood Flow Metab. 25, 1288–1300.

Deutsch, A., Jaronkin, A., Pevzner, E., Mayevsky, A., 2004. BiOS 2004: optical fibers and sensors for medical applications: IV. Real time evaluation of tissue vitality by monitoring of microcirculatory blood flow, HbO_2, and mitochondrial NADH redox state. Proceedings of SPIE, San Jose, California, USA, pp. 5316–5317.

Dirnagl, U., Kaplan, B., Jacewicz, M., Pulsinelli, W., 1989. Continuous measurement of cerebral cortical blood flow by laser-Doppler flowmetry in a rat stroke model. J. Cereb. Blood Flow Metab. 9, 589–596.

Dora, E., 1985. Further studies on reflectometric monitoring of cerebrocortical microcirculation. Importance of lactate anions in coupling between cerebral blood flow and metabolism. Acta Physiol. Hung. 66, 199–211.

Dora, E., Gyulai, L., Kovach, A.G., 1984. Determinants of brain activation-induced cortical NAD/NADH responses in vivo. Brain Res. 299, 61–72.

Feldmeier, J.J. (Ed.), 2003. Hyperbaric Oxygen: Indications And Results; The Hyperbaric Oxygen Therapy Committee Report. Undersea and Hyperbaric Medical Society.

Gill, A.L., Bell, C.N., 2004. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM 97, 385–395.

Haberl, R.L., Heizer, M.L., Marmarou, A., Ellis, E.F., 1989. Laser-Doppler assessment of brain microcirculation: effect of systemic alterations. Am. J. Physiol. 256, H1247–H1254.

Harbig, K., Chance, B., Kovach, A.G., Reivich, M., 1976. *In vivo* measurement of pyridine nucleotide fluorescence from cat brain cortex. J. Appl. Physiol. 41, 480–488.

HBO approved indications. Internet communication.

Ince, C., Coremans, J.M.C.C., Bruining, H.A., 1992. *In vivo* NADH fluorescence. In: Erdmann, W., Bruley, D.F. (Eds.), Adv. Exp. Med.: Oxygen Transport to Tissue XIV. Plenum Press, New York, pp. 277–296.

Jobsis, F.F., Stainsby, W.N., 1968. Oxidation of NADH during contractions of circulated mammalian skeletal muscle. Respir. Physiol. 4, 292–300.

Jobsis, F.F., O'Connor, M., Vitale, A., Vreman, H., 1971. Intracellular redox changes in functioning cerebral cortex. I. Metabolic effects of epileptiform activity. J. Neurophysiol. 3465, 735–749.

Kirkeby, O.J., Rise, I.R., Nordsletten, L., Skjeldal, S., Hall, C., Risoe, C., 1995. Cerebral blood flow measured with intracerebral laser-Dopplerflow probes and radioactive microspheres. J. Appl. Physiol. 79, 1479–1486.

Kramer, R.S., Pearlstein, R.D., 1979. Cerebral cortical microfluorometry at isosbestic wavelengths for correction of vascular artifact. Science 205, 693–696.

Mathews, C.K., Van holde, K.E., Ahern, E.G., 1999. Protein function and evolution. In: Mathews, C.K., Van holde, K.E., Ahern, E.G. (Eds.), Biochemistry. Wesley Longman, pp. 212–256.

Mayevsky, A., 1975. The effect of trimethadione on brain energy metabolism and EEG activity of the conscious rat exposed to HPO. J. Neurosci. Res. 1, 131–142.

Mayevsky, A., 1978a. Shedding light on the awake brain. In: Dutton, P.L., Leigh, J., Scarpa, A. (Eds.), Frontiers in Bienergetics: From Electrons to Tissues. Academic Press, New York, pp. 1467–1476.

Mayevsky, A., 1978b. The responses of an awake brain to HPO under increased CO_2 concentration. In: Silver, I.A., Erecinska, M., Bicher, H.I. (Eds.), Oxygen Transport to Tissue III. Plenum Press, New York, pp. 735–740.

Mayevsky, A., 1983. Multiparameter monitoring of the awake brain under hyperbaric oxygenation. J. Appl. Physiol. 54, 740–748.

Mayevsky, A., 1984. Brain NADH redox state monitored *in vivo* by fiber optic surface fluorometry. Brain Res. Rev. 319, 49–68.

Mayevsky, A., Chance, B., 1982. Intracellular oxidation–reduction state measured in situ by a multichannel fiber-optic surface fluorometer. Science 217, 537–540.

Mayevsky, A., Rogatsky, G.G., 2007. Mitochondrial function *in vivo* evaluated by NADH fluorescence: from animal models to human studies. Am. J. Physiol., Cell Physiol. 292, C615–C640.

Mayevsky, A., Shaya, B., 1980. Factors affecting the development of hyperbaric oxygen toxicity in the awake rat brain. J. Appl. Physiol. 49, 700–707.

Mayevsky, A., Jamieson, D., Chance, B., 1974. Oxygen poisoning in unanesthetized brain: correlation of pyridine nucleotide redox state and electrical activity. Brain Res. 76, 481–491.

Mayevsky, A., Wrobel-Kuhl, K., Mela, L., 1980. High pressure oxygenation in unanesthetized brain: mitochondrial activity, pyridinenucleotide redox state, and electrical activity. Neurol. Res. 1, 305–311.

Mayevsky, A., Zarchin, N., Yoles, E., Tannenbaum, B., 1986. Oxygen supply to the brain in hypoxic and hyperoxic conditions. In: Nicolau, C. (Ed.), Advances in the Biosciences. . O_2-Transport in Red Blood Cells, vol. 54. Pergamon Press, Oxford, New York, pp. 119–132.

Mayevsky, A., Ventura, V., Zarchin, N., 1989. Metabolic responses to hyperbaric oxygenation in the normoxic and ischemic brain. In: Bitterman, N., Lincoln, R. (Eds.), Cerebral Ischemia and Cerebral Resuscitation, Israel Navy, Eilat, Israel, pp. 102–107.

Mayevsky, A., Frank, K., Muck, M., Nioka, S., Kessler, M., Chance, B., 1992. Multiparametric evaluation of brain functions in the Mongolian gerbil in vivo. J. Basic Clin. Physiol. Pharmacol. 3, 323–342.

Mayevsky, A., Meilin, S., Manor, T., Ornstein, E., Zarchin, N., Sonn, J., 1998. Multiparametric monitoring of brain oxygen balance under experimental and clinical conditions. Neurol. Res. 20, S76–S80.

Mayevsky, A., Manor, T., Pevzner, E., Deutsch, A., Etziony, R., Dekel, N., 2002. Real time optical monitoring of tissue vitality *in vivo*. SPIE 4616, 30–39.

Mayevsky, A., Manor, T., Pevzner, E., Deutsch, A., Etziony, R., Dekel, N., Jaronkin, A., 2004. Tissue spectroscope: a novel *in vivo* approach to real time monitoring of tissue vitality. J. Biomed. Opt. 9, 1028–1045.

Moskvin, A.N., Zhilyaev, S.Y., Sharapov, O.I., Platonova, T.F., Gutsaeva, D.R., Kostkin, V.B., Demchenko, I.T., 2003. Brain blood flow modulates the neurotoxic action of hyperbaric oxygen via neuronal and endothelial nitric oxide. Neurosci. Behav. Physiol. 33, 883–888.

Omae, T., Ibayashi, S., Kusuda, K., Nakamura, H., Yagi, H., Fujishima, M., 1998. Effects of high atmospheric pressure and oxygen on middle cerebral blood flow velocity in humans measured by transcranial Doppler. Stroke 29, 94–97.

Rampil, I.J., Litt, L., Mayevsky, A., 1992. Correlated, simultaneous, multiple-wavelength optical monitoring in vivo of localized cerebrocortical NADH and brain microvessel hemoglobin oxygen saturation. J. Clin. Monit. 8, 216–225.

Sato, T., Takeda, Y., Hagioka, S., Zhang, S., Hirakawa, M., 2001. Changes in nitric oxide production and cerebral blood flow before development of hyperbaric oxygen-induced seizures in rats. Brain Res. 918, 131–140.

Scheufler, K.M., Rohrborn, H.J., Zentner, J., 2002. Does tissue oxygen-tension reliably reflect cerebral oxygen delivery and consumption? Anesth. Analg. 95, 1042–1048 (table).

Thom, S.R., Bhopale, V., Fisher, D., Manevich, Y., Huang, P.L., Buerk, D.G., 2002. Stimulation of nitric oxide synthase in cerebral cortex due to elevated partial pressures of oxygen: an oxidative stress response. J. Neurobiol. 51, 85–100.

Tsai, A.G., Johnson, P.C., Intaglietta, M., 2003. Oxygen gradients in the microcirculation. Physiol. Rev. 83, 933–963.

van Hulst, R.A., Haitsma, J.J., Klein, J., Lachmann, B., 2003. Oxygen tension under hyperbaric conditions in healthy pig brain. Clin. Physiol. Funct. Imaging 23, 143–148.

Yoles, E., Zurovsky, Y., Zarchin, N., Mayevsky, A., 2000. The effect of hyperbaric hyperoxia on brain function in the newborn dog *in vivo*. Neurol. Res. 22, 404–408.

Zhang, J., Sam, A.D., Klitzman, B., Piantadosi, C.A., 1995. Inhibition of nitric oxide synthase on brain oxygenation in anesthetized rats exposed to hyperbaric oxygen. Undersea Hyperb. Med. 22, 377–382.

Zhilyaev, S.Y., Moskvin, A.N., Platonova, T.F., Gutsaeva, D.R., Churilina, I.V., Demchenko, I.T., 2003. Hyperoxic vasoconstriction in the brain is mediated by inactivation of nitric oxide by superoxide anions. Neurosci. Behav. Physiol. 33, 783–787.