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A method for measuring brain partial pressure of oxygen in unanesthetized unrestrained subjects: the effect of acute and chronic hypoxia on brain tissue PO_2

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Abstract

The level of tissue oxygenation provides information related to the balance between oxygen delivery, oxygen utilization, tissue reactivity and morphology during physiological conditions. Tissue partial pressure of oxygen (PtO_2) is influenced by the use of anesthesia or restraint. These factors may impact the absolute level of PtO_2. In this study we present a novel fibre optic method to measure brain PtO_2. This method can be used in unanesthetized, unrestrained animals, provides absolute values for PO_2, has a stable calibration, does not consume oxygen and is MRI compatible. Brain PtO_2 was studied during acute hypoxia, as well as before and after 28 days of high altitude acclimatization. A sensor was chronically implanted in the frontal cortex of 8 Wistar rats. It is comprised of a fiber optic probe with a tip containing material that fluoresces with an oxygen dependent lifetime. Brain PtO_2 declines by 80% and 76% pre- and post-acclimatization respectively, when the fraction of inspired oxygen declines from 0.21 to 0.08. In addition, a linear relationship between brain PtO_2 and inspired O_2 levels was demonstrated r^2=0.98 and r^2=0.99 (pre- and post-acclimatization). Hypoxia acclimatization resulted in an increase in the overall brain PtO_2 by approximately 35%. This paper demonstrates the use of a novel chronically implanted fibre optic based sensor for measuring absolute PtO_2. It shows a very strong linear relationship in awake animals between inspired O_2 and tissue O_2, and shows that there is a proportional increase in PtO_2 over a range of inspired values after exposure to chronic hypoxia.

Keywords

Brain; high altitude; oxygen; partial pressure of oxygen; hypoxia; unrestrained; anesthesia

1. Introduction

Tissue PO_2 (PtO_2) provides information on tissue oxygenation, which is determined by the balance between oxygen delivery and utilization. The PtO_2 reports values in the interstitial

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fluid along the diffusion gradient between the capillaries (oxygen supply) and the site of oxygen utilization (the mitochondria).

An adequate supply of oxygen is critical for the cell survival in the brain. The measurement of brain PtO_2 provides a quantifiable measure of oxygenation which has been related to cellular viability in the brain (Dings et al., 1996; Dings et al., 1998) providing important information related to the outcome and prognosis, and monitoring of PtO_2 could provide useful information on the health status of the brain (Dings et al., 1998; Stiefel et al., 2005; Stiefel et al., 2006). PtO_2 has been used to provide insight into the effect on oxygenation of alterations in such variables as hemoglobin p50, hematocrit, angiogenesis, vascular density, alterations in metabolic rate and adaptation to hypoxia (Dunn et al., 2000; Plock et al., 2005; Rascon and Harrison, 2005; Grinakovskaya et al., 2007). Oxygen levels are related to sensitivity to radiation (Lee et al., 1996), thus, PtO_2 values have been used to study tumor oxygenation as a measure of predicting treatment response, and tumor growth (Hou et al., 2009; Khan et al., 2009).

Several non-pathological factors can affect brain oxygenation including anesthesia (Hoffman et al., 1997; Lowry and Fillenz, 2001; Hou et al., 2003), temperature (Gupta et al., 2002; Zhang et al., 2002), emotional stress (Lasbennes et al., 1986; Paisansathan et al., 2007) and neuronal functional activation (Leniger-Follert and Lubbers, 1976; Lowry et al., 1997). Although, the measurement of brain PtO_2 has become a very important tool, most studies of healthy brain PtO_2 have been undertaken either under anesthesia or during physical restraint, which may influence the results. Also, the act of implanting an electrode or probe will cause vascular disruption, which may influence the PtO_2.

Chronic implants provide significant advantages over acute measurements where the sensor is implanted and measurements taken within a day. Some of these approaches allow for measurements to be taken hours to weeks after the implant. This time period allows tissue trauma to heal, although the cell composition may vary from pre-implant conditions (Polikov et al., 2005). It also allows for the measurements to be taken in the same location and, depending on the method, can be done without anesthesia. Methods have been developed that allow measurement of PtO_2 in awake and unrestrained subjects over longer periods (Lowry et al., 1997; Dunn et al., 2000; Lowry and Fillenz, 2001; Ma and Wu, 2008; Bazzu et al., 2009; Ma et al., 2009).

In this paper, we present a new method whereby measurements of absolute values of PtO_2 can be obtained using a chronically implantable fiber combined with an optical fluorescence method.

Although it is possible to obtain absolute quantification with polarographic electrodes, these consume oxygen by the electro-chemical reduction reaction (Clark et al., 1958; McLaurin and Nichols, 1959; Cooper, 1963; Holmstrom et al., 1998; El-Deab and Ohsaka, 2003). The calibration may also drift over time and so it is common to report results in terms of delta milli-amperage instead of PtO_2. Good examples of the use of electrodes in awake animal models include (Lowry et al., 1996; Lowry et al., 1997; Ma and Wu, 2008; Bazzu et al., 2009; Ma et al., 2009).

The use of fluorescence based fiber optic probes has advantages when compared with other methods. Fluorescent detection involves quantification of a quenching function which does not consume oxygen (Griffiths and Robinson, 1999). The calibration is, by definition, stable over time, and the detectors can be pre-calibrated at the manufacturer. The optical nature of the method means that fiber optics can be used. The glass shaft and other materials are biocompatible and the probes can be used in combination with other imaging devices (such

as MRI). This paper reports a design that can be chronically implanted into tissue, and measurements obtained over weeks.

We measured PtO_2 in brain during and after exposure to acute and chronic systemic hypoxia to show proof of principle, as well as to add to our knowledge of the response of brain oxygenation to hypoxia. Hypoxia exposure will stimulate adaptations in oxygen delivery to the brain that allow for survival during conditions of low oxygen. Such adaptations are known to include an increase in hemoglobin concentration (Heinicke et al., 2003), hematocrit (LaManna, 1992), polycythemia (Jha et al., 2002) and vascular density (LaManna, 1992). This remodeling will result in systematic changes in brain PtO_2 (Dunn et al., 2000). It is likely that chronic hypoxia can occur in brain during a range of conditions including cancer progression, high altitude exposure, anemia, vascular disease and long term artificial ventilation.

In this study, a chronically implantable fiber optic probe was developed. This paper describes its use in detail, and applies it to show how PtO_2 varies in rat brain with acute and chronic exposure to hypoxia.

2. Material and methods

2.1. Animals

Male Wistar rats (150 - 200g) were obtained from Charles River Laboratories (Wilmington, MA). Temperature was maintained at 22 ± 1 °C and light was on a 12 hr diurnal cycle. Rats were fed food and water *ad libitum*. Studies were done in accordance with the guidelines for the care and use of laboratory animals. The animal protocol was reviewed and approved by the University of Calgary Animal Care Committee.

2.2. Animal preparation

Animals were routinely handled (90 minutes/day, five times/ week) for at least two weeks before studies to reduce the stress of handling during the measurements. During implantation, anesthesia was induced with 5% and maintained at 1.5-2 % isofluorane (Aerrane, Isofluorane, Baxter Corporation) with 30% oxygen and the balance nitrogen delivered through a nosecone. Oxygen saturation, heart rate, respiration rate, breath and pulse distention were monitored during surgery using a multiparameter monitor (Mouse Ox small animal oximeter, STARR® Life Science Corp.). Core temperature was maintained at 37 ± 1°C using a servo-controlled temperature regulator (Cole-Parmer Instrument Company, VH, USA). After shaving the back of the head, a midline incision of about 2-3 cm was made in the skin over the skull. The skin was retracted to the side. In a small section, hypodermis and soft tissues were scraped away. A 1.5 mm diameter burr hole was drilled 2 mm lateral sagittal suture and 3 mm caudal to the bregma, until the external meningeal layer was visible. Three more superficial holes were drilled 5 mm away from the probe location. Several superficial channels were carved on the skull surface, for the foundation of dental cement (Ortho-Jet, Lang, Dental Manufacturers, Co, Inc, Wheeling, Il, US). In the central hole, the external meningeal layer was breached using a bevel-tip hypodermic needle (30.5G). This was done in order to minimize stress on the fiber optic tip when inserting into the brain. The custom made fiber optic tip was inserted, and then retracted by approximately 0.1mm to its final position to reduce compression of the tissue (Jensen et al., 2006; Stice and Muthuswamy, 2009). The probe was secured with a bridge of dental cement and three 1mm non-magnetic MRI compatible screws (Plastics 1 One, Inc, Roanoke, VA). The incision was sutured closed using Vicryl 3/0 (Ethicon®) and topical antibiotic cream applied. Buprenorphine (0.5 mcg/ 1000g) was subcutaneously administrated for pain relief, followed by oral Buprenorphin twice a day for 3 days.

2.3. Brain PtO_2 measurements

Brain PtO_2 was measured with the Oxylite system (Oxford Optronics, UK) (Fig. 1) using a fiber optic probe that was re-designed for this project for chronic implantation in tissue (Griffiths and Robinson, 1999). The principle of operation is based on oxygen quenching the fluorescence generated in a dye (platinum (II) meso-tetra (pentafluorophenyl) porphine) embedded into the tip of the probe by an optical pulse in an oxygen dependent fashion. The probes are pre-calibrated by Oxford Optronics in solutions of multiple temperatures and PO_2 values. Although factors such as pH sensitivity have not been measured, the *in vivo* results compare favorably with electrode measurements of PO_2, even in tumors where pH can vary considerably (Seddon et al., 2001;Wen et al., 2008). The chronically implantable fiber optic probe was constructed by Oxford Optronics based on a design supplied by Dr. Dunn. The probe was constructed using a teflon holder where a fiber optic protrudes to the desired depth in the tissue. In this case, the tip was 1.8mm in length, and 250μm in diameter. The probe was secured to the skull under surgery. When studies are undertaken, a longer fiber optic was used to connect the implantable probe to the Oxylite using a teflon tubular sleeve.

During measurements, rats were placed inside a 10 liter chamber (25×20×20cm), while awake and freely moving. During acclimation and during measurements, environmental enrichment and food were placed inside the chamber in order to keep the animals occupied and to maintain the environment as closely as possible to that which was normal for the animals

Brain partial pressure of oxygen (PtO_2) was recorded every second (1 Hz) during 10 minutes at each FiO_2. Analogue output signals are accessed via 2 pin male d-connectors located on the back of the Oxylite system and digitized using the Biopac system (BIOPAC Systems, Inc. CA, USA).

2.4. Induction of acute and chronic hypoxia

Brain PtO_2 was measured during normoxia and acute hypoxia 7 days after the probe implantation in 8 rats. Experiments were performed when the rats were awake, non-sedated and freely moving. Hypoxia was induced in the chamber by changing the fraction of inspired oxygen (FiO_2) in the inflowing gas from 0.21 to 0.08 using a mass flow controller. (Brooks Instruments, Mass flow controller 5850 series E). Chamber PO_2 was measured with an additional fiber optic probe when the chamber PO_2 was less than 90mmHg (the approximate upper range of the sensor). Above this range the inspired PO_2 was calculated from FiO_2 taking into account the barometric pressure measured with a mercury manometer.

Since CO_2 may influence cerebral blood flow and brain PtO_2, the fraction of inspired carbon dioxide ($FiCO_2$) inside the chamber was also recorded using a CO_2 gas analyzer (CO_2 analyzer CA-ZA Sable systems international). No significant increment was detected in the chamber after almost 2 hours of experimentation.

Brain PtO_2 was recorded while the animals breathed 13 different, declining levels of FiO_2 for 10 minutes each. On average, 6 minutes were necessary for the chamber gasses to reach a steady state after changing the gas mixture and so only the last 5 minutes of data were averaged for further analysis.

After the acute hypoxia study, 6 rats (2 excluded because of probe detachment) were placed in a custom built hypobaric chamber for acclimatization to chronic hypoxia. The rats were exposed to 375±2 mmHg of barometric pressure which is close to about 50% of inspired O_2 at sea level. The average barometric pressure in Calgary was 666±1 mmHg during the whole study. After 28 days of acclimatization, brain PtO_2 was measured again during the range of

inspired O_2 values that was used to study acute hypoxia before acclimatization. This was done in order to compare the response to acute hypoxia from pre to post acclimatization.

2.5. Post-hypoxia MRI assessment

After the completion of the experiment, MRI was used to verify probe placement and to investigate potential tissue damage and edema (Fig. 2). A gradient and spin echo MRI T1 and T2 weighting (9.4T) were performed under anesthesia (2-2.5% isofluorane). The brain was imaged as 15 slices (1 mm each) for regional evaluation of scarring, hemorrhage, edema or necrosis that may be associated with probe placement (Hoopes et al., 1997).

2.6. Histology

Wistar rats were anesthetized with intraperitoneal ketamine/xylazine at a dose of 10 mg/100g body weight (Bimeda-MTC Animal Health Inc., Cambridge, ON, Canada). Cardiac perfusion into the ascending aorta was carried out with 250 ml of cold normal saline followed by 150 ml of 10% neutral buffered formalin solution (Sigma-Aldrich Inc., St. Louis, MO, USA). The brain was removed, immersed in 10% formalin solution for 2-3 days at room temperature, and then washed in phosphate buffered saline (pH 7.4). This was followed by embedding in paraffin wax. Consecutive coronal sections (6 μm) were cut and stained for hematoxylin and eosin.

3. Results

3.1. Calibration and temperature sensitivity

A subset of probes was tested for temperature sensitivity. The tip of the fiber optic probe was immersed in a beaker with distilled water at 37 °C while 3.6% oxygen was bubbled in the solution. The temperature was increased in a step wise fashion and one hour of stable PO_2 data was obtained at each temperature. The temperature correction was either entered automatically, or it was manually set to remain at 37 °C. The difference in the PO_2 measured from 34 °C to 42 °C with and without temperature compensation was not significant (paired t-test, $p<0.05$) (24.1 ± 0.30 vs. 24.3 ± 0.42 mmHg respectively, mean±SD.) In a parallel study, the probes were retested in saline after 20 days of implantation and the PO_2 values were also not significantly different, confirming that the calibration is stable.

3.2. MRI and histological assessment

All probe tip locations were confirmed using a 9.4 T MRI to be within the cortex and not in the white matter. The MRI's also confirmed that the probes are MRI compatible and generate minimal artifacts (Fig. 2). There were no signs of edema around the probe as indicated by the lack of enhancement on T2w MRI. There was no collection of blood as indicated by the absence of hyperintense regions. The H&E stained sections showed no significant signs of inflammation (Fig. 2) or gliosis, which is often detected by H&E staining (Tihan et al., 2001;Polikov et al., 2005;Gonul et al., 2007).

3.3. In-vivo rat cortex PtO_2

Although stress indices such as serotonin serum levels were not measured, normal behaviours such as drinking, eating, sleeping and exploring the cage were seen throughout the experiment. These behaviors suggest that the animals were experiencing minimal stress (Hori et al., 2004; Miyake et al., 2005).

PtO_2 values were measured immediately on implant, 1 hour later while under anesthesia and after recovery. In general, the PtO_2 values were low immediately after implant. Brain PtO_2 values start rising after 5 minutes. The data obtained during surgery (2% isofluorane) was,

on average, about 25% lower than the data obtained 4-5 days post-surgery in the animals used for acclimation (22.5±2.1 vs. 30.2±3.3 mean±SE).

Brain PtO_2 was measured at 2 weeks post implant, and again between 3-5 weeks later in a control group of awake and unrestrained rats. Brain PtO_2 values did not show any significant change over time (paired t-test, $p>0.05$, $n=4$) with the values being 25.8±3.4 mmHg (mean ±S.E.) and 24.5±2.56 mmHg respectively.

3.4. Brain PtO_2 during acute hypoxia before and after acclimatization

Brain PtO_2 in the pre-acclimatized subjects, breathing 21% oxygen (139 mmHg), was 30.2±3.3 mmHg (mean ± SE, n=8), while after 28 days of chronic hypoxia brain PtO_2 at the same FiO_2 was 39.2±5.5 mmHg (mean±SE, n=6). This was a 30% increase. Brain PtO_2 increased over the range of inspired O_2 values on average by 35% after acclimatization.

Acute hypoxia caused a decline in PtO_2 within seconds, with an average reduction of 76% when FiO_2 was reduced from 0.21 to 0.08 (62%). Although the slopes appear non-linear at the lower values, one can generalize that over the range of FiO_2 from 0.21 to 0.10 the slopes are approximately linear. The slope of the regression (PtO_2 = a (inspired O_2) + b) was 0.31 before acclimatization and 0.37 after acclimatization (Fig. 5). A paired t-test comparing the slope of inspired O_2 vs. PtO_2 showed a significant difference between acclimatized and un-acclimatized rats ($p \leq .0.001$).

A two-way ANOVA was used to test the combined effect of acclimatization and the changes with acute hypoxia. A significant effect was found on PtO_2 with acclimatization ($p < 0.01$). As there was no interaction between the two factors ($p = 0.93$), a Holm-Sidak post-hoc method was used to evaluate differences among the means before and after acclimatization at the different values of FiO_2 (Fig. 5).

At the completion of the study (after acclimatization and the final measurements in acute hypoxia), approximately 15 minutes after breathing the lowest level of oxygen, brain PtO_2 was measured again in awake rats while breathing 21%. Following this, the subjects were anesthetized with 5% isofluorane and PtO_2 was re-measured. The brain PtO_2 significantly increased after breathing 5% isofluorane, from 39.5±4.3 mmHg while awake, to 49.05±4.3 mmHg (mean±SE) approximately 2-3 minutes after induction of anesthesia.

4. DISCUSSION

4.1. The measurement of PO_2

A wide range of methods have been used to measure PtO_2. For a review see Swartz, Dunn (Swartz et al., 2003). Table 1 shows a range of examples. Different technologies and methods have been used including electrodes, electron paramagnetic resonance, and fluorescent materials with nuclear magnetic resonance (NMR).

Chronic measurements have also been previously undertaken. Clarke implanted electrodes for up to two years (Clark et al., 1958). They reported values as a depolarizing current in unanesthetized cat brain, and showed that current declines with reduced inspired O_2. An implant system was developed that allowed for a commercially available PO_2 electrode to be inserted and removed as needed (Ma and Wu, 2008; Ma et al., 2009). This allows for chronic measurements using a standard electrode. The electrode is inserted through a guide cannula for each session of measurements. It is possible that this electrode will damage blood vessels around the tip with each insertion, which may, in part, explain why it requires about 1hr after insertion to achieve a stable baseline (Ma et al., 2009). A carbon paste electrode was used with differential pulse amperometry to measure changes in current as a

correlate of changes in brain PtO_2 in a range of conditions (Lowry et al., 1996; Lowry et al., 1997; Lowry and Fillenz, 2001). An amperometric electrode with a self contained power source has been implanted subcutaneously in rabbits and PO_2 monitored up to 5 days post implant (Ward et al., 2002). Constant potential amperometry has been used with an implanted silver electrode connected to a carbon microsensor and a telemetry circuit (Bazzu et al., 2009). Calibrations were done before and after 8 days of implant. Most of the data were reported in delta amps and there was a small but statistically significant drift in current.

The usage of platinum based fluorescence in fiber optic probes for the measurement of brain oxygenation has certain advantages over other methods. In particular, the fluorescence calibration is dependent on the rate of quenching of fluorescence, and so the calibration remains stable over the lifetime of the sensor. Previously, we compared the manufacture's pre-calibration with an in-house calibration of a ruthinum based sensor and, although the values differed by a small amount, the results were consistent and very precise below approximately 40mmHg (Nwaigwe et al., 2003). Another advantage of fluorescent probes is that the sensitivity improves as PO_2 declines. The sampling volume is related to the tip diameter and will be similar to other probes of similar diameter. One estimate is that the probes are capable of sampling a volume of tissue of 0.25 - 0.35 mm^3 (Griffiths and Robinson, 1999). The sensors do not use O_2 unlike electrode methods, and so will not perturb the local O_2 environment. There is no time required to stabilize the polarization current, unlike electode systems which may require up to 47 minutes to stabilize (Bazzu et al., 2009). Disadvantages include the problem that the fluorescent material will "photobleach" over time, which means that the signal/noise will decline. Also, the fibre optic probes are fragile and care is needed during insertion.

Previous probes used a ruthenium based fluorescence material. The calibration was found to have significant temperature variation (Nwaigwe et al., 2003). The current version using the platinum based material showed little variation in PO_2 over an 8°C range of temperature. This is important in this study as body temperature and brain temperature will decline during acute hypoxia (Ma et al., 2009; Natah et al., 2009). The relative insensitivity of the probe to temperature allowed the study to proceed without the need for an additional implant to record and compensate for changes in temperature.

With this type of probe, brain PtO_2 can be measured in unanesthetized and unrestrained animals during long term chronic studies. The current study was designed to minimize any potential effects of obtaining measurements during acute trauma at the implant site. By this, we mean measurements were only taken days after implantation. This is a potential advantage over a chronic implant method that involves removal and reinsertion of the electrodes (Ma and Wu, 2008). Such a method may cause local trauma upon insertion. Histology indicates that there is no bleeding or inflammation but does not rule out an abnormal distribution of cells as might be expected with chronic implants (Polikov et al., 2005). The use of these probes also avoids the effects of anesthesia (Lasbennes et al., 1986; Hoffman and Edelman, 2000; Gupta et al., 2002).

The probes are made of optical fibers and are therefore compatible with imaging techniques such as MRI and CT scans, a great advantage over metal based sensors (Holmstrom et al., 1998; El-Deab and Ohsaka, 2003). The probes are insensitive to motion (breathing or ventilation) if the probe is positioned and secured in such a way as to ensure no relative motion between the probe and surrounding tissue. Due to the fact that quench rates are lower at lower PO_2 values, the precision of the measurement increases during hypoxia and is less than 1mm Hg below 30 mmHg. Although signal/noise will decline over time, the calibration is insensitive to photobleaching, and ambient light (Griffiths and Robinson, 1999). Another important feature is that brain PtO_2 is quantified in absolute units (mmHg or kPa). The use

of absolute units allows for comparison between subjects, unlike the method of calibration in units of delta current (Travis and Clark, 1965; Lowry et al., 1997; Lowry and Fillenz, 2001). These features allow us to rapidly measure brain PtO_2 during physiological conditions, avoiding the need for re-calibration (Lowry et al., 1997; Lowry and Fillenz, 2001; Ma and Wu, 2008; Bazzu et al., 2009).

The tissue response to a fiber optic implant should be roughly similar to the response caused by other type of needle-shape sensor. However, the shape and the texture of the Oxylite probes may be associated with milder tissue response (Polikov et al., 2005). We did not observe any major histological sign of acute or chronic inflammation. Although H&E staining is not specific for gliosis, significant gliosis and degenerative changes around the probe should be detectable (Gonul et al., 2007; Stice and Muthuswamy, 2009). It is useful to note that PtO_2 in a control group of subjects did not change over many weeks. This indicates that should gliosis occur as it may do over weeks around an implant (Polikov et al., 2005) the impact on average PtO_2 is likely to be minor.

The time required between implant and stable readings of PtO_2 is not certain. Studies with implants of crystals of lithium phthalocyanine, which use electron paramagnetic resonance to detect PtO_2 suggest that 1-3 days are needed before the PtO_2 readings stabilize (Liu et al., 1995; Dunn and Swartz, 2003). Clark suggested that 1-3 weeks was required before current stabilized in electrodes implanted in brain (Clark et al., 1958). Some have shown that 24 hrs is sufficient time post implantation of an oxygen sensor to reach a stable, reproducible value for PtO_2 (Liu et al., 1995; Lowry et al., 1997; Ward et al., 2002). A study measuring PtO_2 in brain over 8 days found no significant difference between days, but did not report the error of the measurements (Bazzu et al., 2009) or the time of first measurement. Histological studies showed that 7 days allowed time to allow time for mechanical trauma associated with the implantation to heal (Polikov et al., 2005), although one has to recognize that there may be histological changes.

In the current study, measurements were initiated 1 week after implantation. Based on the argument above, this provides sufficient time to reach a steady state with respect to tissue remodeling and PtO_2. This was confirmed by the fact that values measured after 1 week did not differ significantly from those measured 6 weeks later. A good feature of most implants is that measurements are obtained when the vascular system has resealed and it is expected that the tissue has healed.

Although it has been shown that anesthesia will change brain PtO_2, (Lei et al., 2001; Lowry and Fillenz, 2001; Hou et al., 2005) these data confirm that isoflurane anesthesia in a spontaneously ventilating animal can result in an increase in brain PtO_2. The brain PtO_2 increased by approximately 26% when the animals were anesthetized with 5% isofluorane when compared to unanesthetized brain PtO_2.

4.2. The response of brain PtO_2 to acute hypoxia

In awake, but restrained Wistar rats, the mean cortical PtO_2 value while breathing 21% oxygen was 26 ± 15 mmHg (Dunn et al., 2000) while in this study it was 30mmHg. Awake arctic ground squirrels had a striatum PtO_2 of 21.2 ± 2.1mmHg measured with a Clark-type electrode and rats had 15mmHg under similar conditions (Ma and Wu, 2008). A range of measured values is shown in Table 1.

During acute hypoxia, the average PtO_2 decline is strongly correlated with a decline in inspired O_2 (Fig. 4). In some animals this decline appears sigmoidal, while in others it is more linear, especially after acclimatization (Fig. 5). It has been known for some time that PtO_2 in brain declines with declining inspired O_2 (Clark et al., 1958;Leniger-Follert and

Lubbers, 1976;Meixensberger et al., 1993;Rolett et al., 2000;Ma et al., 2009). Even so, the current study is unique because of three factors: it was performed on awake and unrestrained animals, it was reproduced with sufficient samples to describe a significant relationship and it shows the impact of long term systemic hypoxia on this relationship. The slope is reproducible, even after an intervention such as exposure to chronic hypoxia, indicates that a slope determined for humans could possibly be used clinically to determine if PtO_2 is within a normal range or responding with an appropriate proportional change for a given inspired O_2 or blood oxygen saturation. This may provide an indication that there is abnormal metabolic rate or neurovascular coupling. Early work by Meixenberger showed that there is a relationship between PaO_2 and PtO_2 in human brain, although the data were not as extensive and the inspired PO_2 was not reported (Meixensberger et al., 1993).Variation between slopes could be a consistent marker of differences in cell to vascular coupling.

These and previous studies show that oxygen content in the brain can range extensively without damage and underscore the premise that PtO_2 is not held constant over the short term. However, at some critical point, the level of oxygen begins to limit function and may correlate with damage. A PtO_2 of 9 mmHg has been correlated with the beginning of energy failure (Rolett et al., 2000). In clinical studies of patients with severe head trauma undergoing surgery, a PtO_2 of less than 10mmHg for an extended period of time has been associated with poor outcome, including death (Bardt et al., 1998). Even so, in 3 of 6 subjects, PtO_2 values significantly less than 10 mmHg were observed.

4.3. The response of brain PtO_2 to chronic hypoxia

Chronic exposure to low oxygen conditions resulted in an increase in brain PtO_2 at all values of inspired O_2. This was first shown in a study measuring brain PtO_2 in animals breathing normobaric 21% O_2 before and after acclimatization to hypoxia (Dunn et al., 2000). Cerebral blood flow (CBF) increases as hypoxia becomes more severe (Kogure et al., 1970; Dahlgren, 1990) despite the fall in PCO_2 (Borgstrom et al., 1975; Beck and Krieglstein, 1987; LaManna, 1992). This increment in CBF plays an important role in increasing PtO_2 during acute hypoxia. During chronic hypoxia at 0.10 FiO_2, CBF begins to return toward the baseline level (Severinghaus et al., 1966; Zhou et al., 2008). This is because, in part, oxygen delivery is improved by increasing oxygen carrying capacity and capillary density (LaManna, 1992; Boero et al., 1999; Dunn et al., 2004).

In a previous study, while breathing normoxic gases, PtO_2 increased by 228% after acclimatization (Dunn et al., 2000). In the current study, the PtO_2 increases on average by 35% (4% to 68% over the range of FiO_2 values). Although the animals were handled routinely in this earlier study, we can not rule out the possibility that they were stressed. If this were the case, CBF may be closer to maximum during the measurements (Ohata et al., 1981). If there was a greater capacity to increase O_2 delivery through increased CBF after acclimatization, then the % difference after acclimatization may be higher based on modeling studies (Grinberg et al., 2005).

Another factor that is very likely to contribute to the difference is that the pre-acclimatized animals were breathing 157 mmHg O_2 in the previous study (Dunn et al., 2000) and 139mmHg in the current study. Altitude acclimatization probably does not occur linearly with altitude. Arterial oxy-hemoglobin saturation in human subjects who had been living at moderate altitude (~1500m) was higher when traveling to altitude than those measured in lowlanders exposed directly to high altitude (Muza et al., 2001). The animals in the current study had lived for 21 days at 1060 m (the altitude of Calgary, AB) before the study and so may be considered to have some altitude acclimation before the study began. Finally, the study design may play a role. In the current study, the FiO_2 was changed slowly, over 60 minutes. In the previous study, FiO_2 was changed within a few seconds from 21% to 10%

O_2. This large acute shift in O_2 could have a larger influence on CBF. In any case, both studies are conclusive in showing that acclimatization to low oxygen results in increased PtO_2 in brain for any given level of inspired O_2.

5. Conclusions

This study describes a modification of the Oxylite fibre optic system, which allows for the measurement of PtO_2 in awake unrestrained subjects using a custom built chronic implant. The feasibility of this method for measuring brain PtO_2 in unanesthetized and unrestrained subjects over weeks of study is reported.

While others have shown that PtO_2 declines with a reduction in inspired O_2, they have included a limited number of points or subjects. To our knowledge, this is the first time enough points and subjects have been measured to accurately describe the relationship between inspired O_2 and brain PtO_2. It is certainly the first time this has been attempted in an unanesthetized and unrestrained animal without the confounding effects of anesthesia.

This method has the advantage that it reports absolute values of PO_2, the calibration is stable over time, the materials are biocompatible, the sensors are compatible with other imaging modalities such as MRI and CT, and the novel probes are now commercially available. This modification of the Oxylite opens the possibility of assessing tissue oxygenation in animal models and in patients in a range of medically relevant conditions including, but not limited to cancer, stroke, epilepsy and wound healing.

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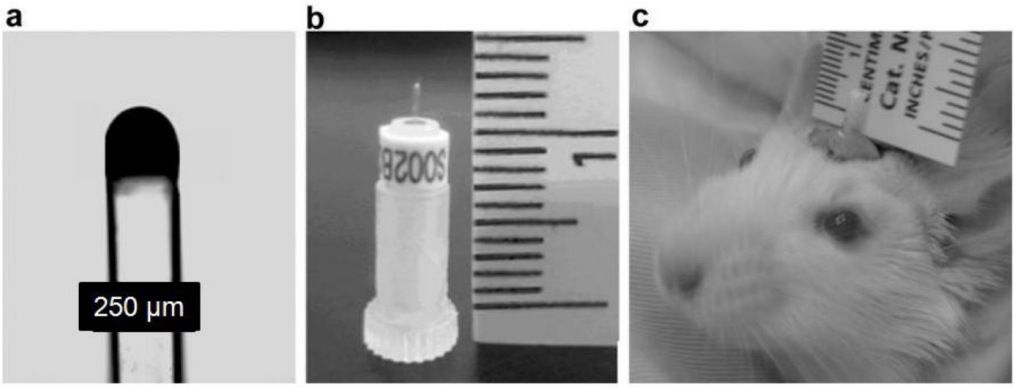

Fig. 1.

The implantable optode **(a)** micrograph of the fiber optic probe (10X). **(b)** Photograph of a chronically implantable probe supported by a transport sleeve. **(c)** Photograph of implanted fiber optic probe showing size, location and the supporting ring of dental cement. The picture was taken when the animal was awake.

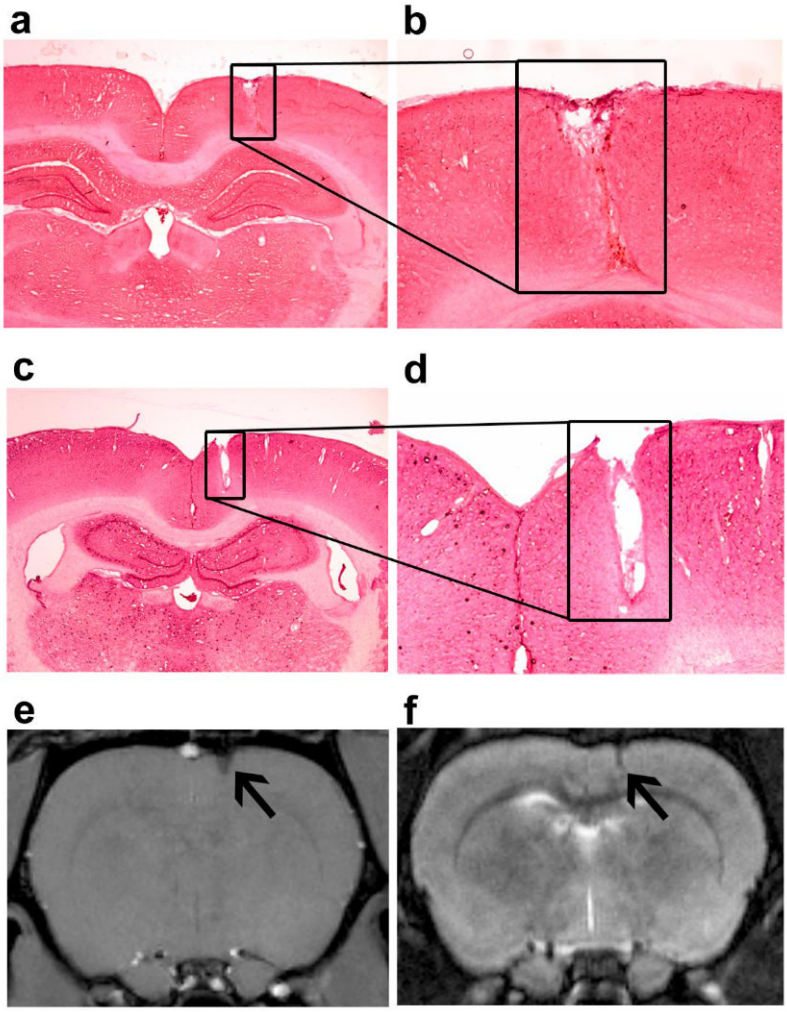

Fig. 2.

Description of implant region. **(a & c)** Coronal sections stained with H&E of the brain of two example subjects (4X magnification). The black square shows the location of the probe in the frontal cortex. **(b & d)** 10X magnification of the probe location. The tip was largely in cortical layers 4-5. There is no sign of inflammation. **(e)** Gradient echo MRI (4 days post-implantation) and **(f)** Spin echo MRI taken 30 days post implantation of the corresponding subject shown above. Black arrows show the location of the probe.

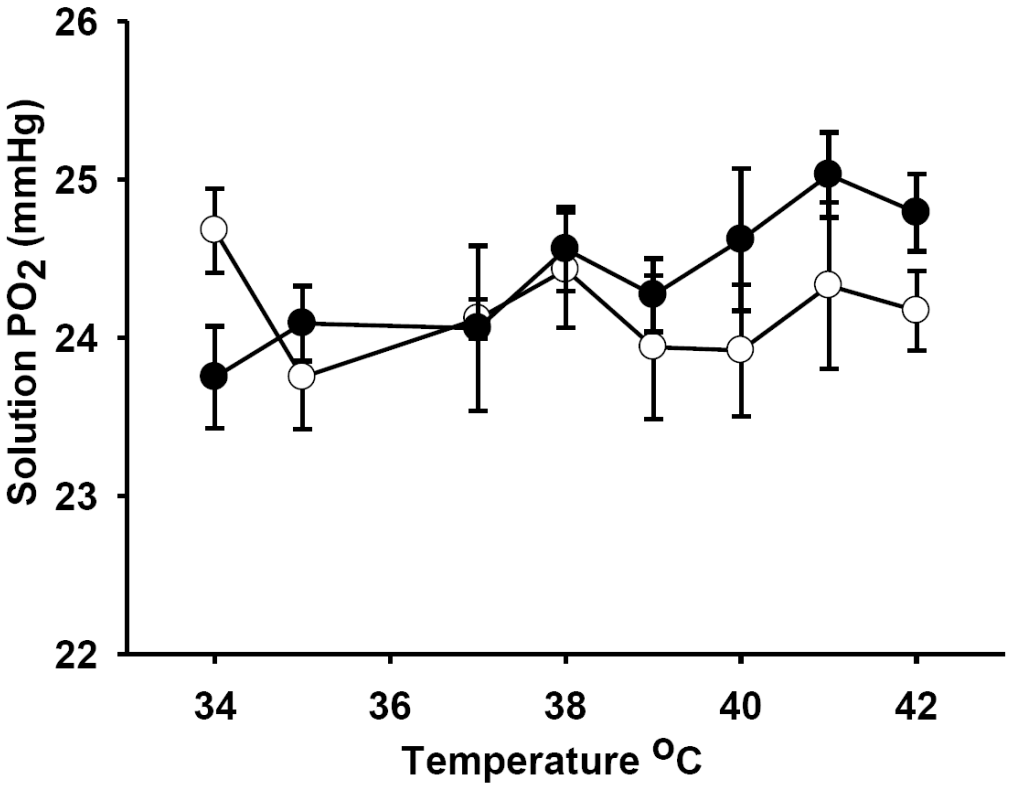

Fig. 3.

Temperature effect on measurements of PO_2. Measurements were taken of the same solution with, and without temperature correction. The probe was immersed in a bath with a fixed PO_2 and the bath temperature ranged from 34-42 °C. The open circles show the PO_2 measured using automatic temperature correction. The closed circles show the PO_2 of the same bath when a fixed input temperature of 37 °C was used. Although there was a drift up, the change was not significant and the absolute difference from an automatic calibration was less than 1mmHg, well within the natural variation seen in a subject.

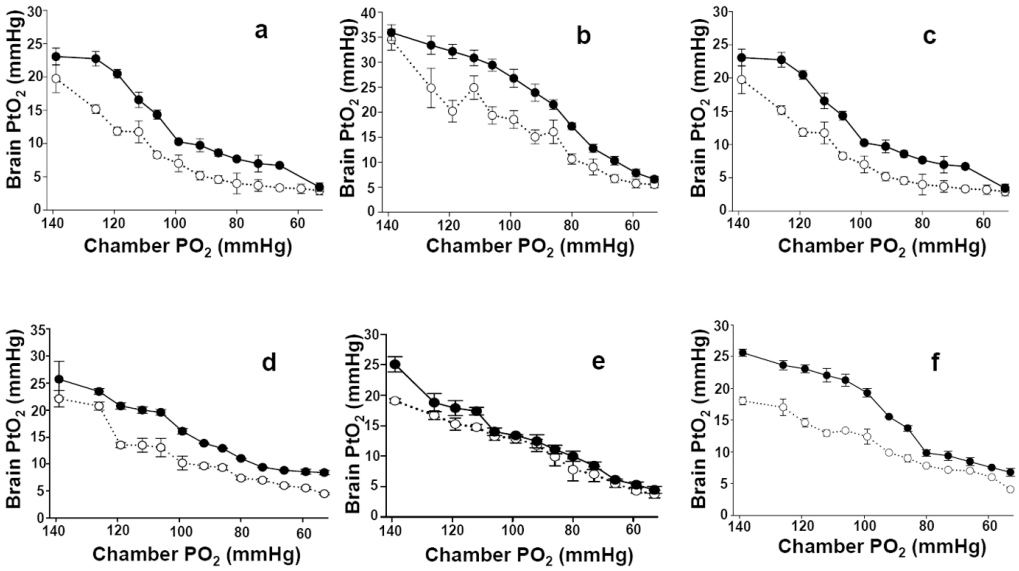

Fig. 4.

Brain PtO_2 measured in awake unrestrained animals during acute hypoxia, before and after acclimatization to hypoxia. White circles are pre-acclimatization and the black circles are after 28 days acclimatization (375 ± 2 mmHg). These data show the degree of subject variability.

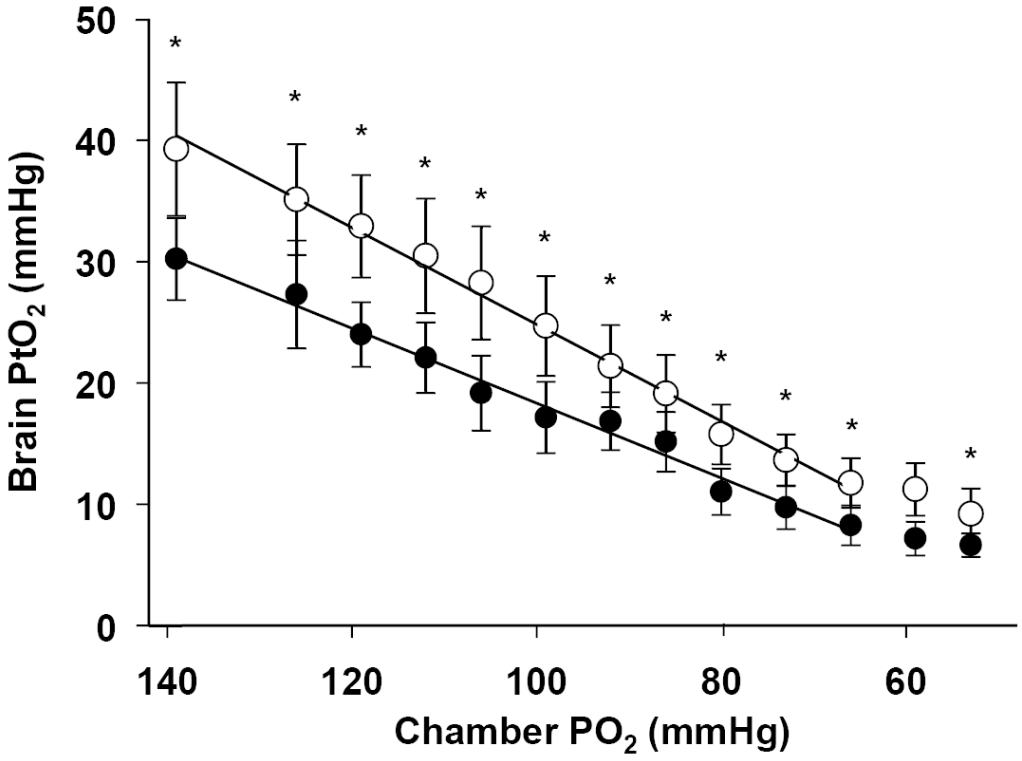

Fig. 5.

The relationship between PtO_2 and inspired O_2 before and after acclimatization. **(a)** A linear regression is fit through the data, not including the final 2 points at low values of inspired O_2. Black circles, pre-acclimatization, r^2=0.98, Open circles, post acclimatization. r^2=0.99. The slopes of the curves before and after acclimatization are 0.31 and 0.37 respectively. The slopes are significantly different ($p<0.001$)(mean ± S.E. $p \leq 0.01$). Two way ANOVA with a Holm-Sidak post-hoc method was used to test for changes at each FiO_2 between pre- and post-acclimatization (* indicates significance, $p < 0.05$).

Table 1

Example data on brain PtO_2 (mean±SD unless noted)

Brain PtO_2 (mmHg)	FiO_2 (%)	Method/ species	Notes	Ref.
14.4±2.5	21%	EPR in Wistar Rats (mean ±SE)	0.8-1.0% Isofluorane	(Hou et al., 2005)
13.7±3			0.7-0.8% Halothane	
6.7±1.9	33%	EPR in Rats (mean±SE)	KetamineXylazine	(Swartz et al., 2003)
13.9±3			Pentobarbital	
16.0±4.5			Urethane/chloralose	
22.6±1.1			1.5% Halothane	
44.6±5.1			2.2% Isoflurane	
26.7±7	26%	EPR in Rats	1.1% Isofluorane	(Lei et al., 2001)
29.6±8			Ketamine	
19±7.8			Ketamine/Xylazine	
26.0±4.8	28%	Oxylite in rats	Control group	(Nwaigwe et al., 2000)
14.8.0±5.2			After 10 min hyperventilation 2% isofluorane	
27.1.0±7.5	21%	EPR in Rats	Awake, before acclimation and after living 4 days at 10% O_2	(Dunn et al., 2000)
49.0±11				
15.1±1.8	30%	EPR in rats (mean±SE)	Ketamine/xylazine	(Rolett et al., 2000)
8.8±0.4	15%			
6.8±0.3	10%			
20.6±10	30%	Gold O_2 sensors in Rats	Urethane (750 mg/kg b.w.) or Pentobarbital (30 mg/kg b.w.)	(Metzger and Heuber, 1977)
14.4±1.6	21%	Polarographic electrodes in rats	Sodium Pentobarbital (50 mg/kg b.w.)	(Weiss et al., 1976)
9.6±1.1	10%			
21.2±2.0	21%	Electrode, Ground Squirrel (mean±SE)	Euthermy, non-sedated, non-anesthetized	(Ma and Wu, 2008)
12.9±0.9	30%	10 μm polarographic platinum electrodes in Wistar rats	Pentobarbitol-sodium 35mg/kg Turbocurarine-hydrochloride 10mg/kg	(Sick et al., 1982)