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Real-time monitoring of ischemic and contralateral brain pO₂ during stroke by variable length multisite resonators

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Abstract

Purpose—Electron paramagnetic resonance (EPR) oximetry using variable length multi-probe implantable resonator (IR), was used to investigate the temporal changes in the ischemic and contralateral brain pO₂ during stroke in rats.

Material and Methods—The EPR signal to noise ratio (S/N) of the IR with four sensor loops at a depth of up to 11 mm were compared with direct implantation of lithium phthalocyanine (LiPc, oximetry probe) deposits in vitro. These IRs were used to follow the temporal changes in pO₂ at two sites in each hemisphere during ischemia induced by left middle cerebral artery occlusion (MCAO) in rats breathing 30% O₂ or 100% O₂.

Results—The S/N ratios of the IRs were significantly greater than the LiPc deposits. A similar pO₂ at two sites in each hemisphere prior to the onset of ischemia was observed in rats breathing 30% O₂. However, a significant decline in the pO₂ of the left cortex and striatum occurred during ischemia but no change in the pO₂ of the contralateral brain was observed. A significant increase in the pO₂ of only the contralateral non-ischemic brain was observed in the rats breathing 100% O₂. No significant difference in the infarct volume was evident between the animals breathing 30% O₂ or 100% O₂ during ischemia.

Conclusions—EPR oximetry with IRs can repeatedly assess temporal changes in the brain pO₂ at four sites simultaneously during stroke. This oximetry approach can be used to test and develop interventions to rescue ischemic tissue by modulating cerebral pO₂ during stroke.

Keywords

cerebral ischemia; reperfusion; stroke; EPR oximetry; implantable resonator; pO₂

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CONFLICT OF INTEREST NOTIFICATION

No conflict of interest.

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INTRODUCTION

Tissue pO_2 is one of the most important factors in various physiological and pathological processes. Therefore, it will be very useful to develop methods for a direct assessment of tissue pO_2 with sufficient sensitivity, accuracy and ease (1,2) to enhance our understanding of pathological processes, such as stroke, which is the foremost cause of long-term disability and mortality in the United States.

Several methods have been developed to measure pO_2 , including the Clark electrode, optical methods based on fluorescence quenching or phosphorescence quenching, MRI, PET and EPR oximetry (3–8). Each of these methods has specific advantages as well as limitations, especially for repeated measurement of cerebral pO_2 (5). Clark electrodes cause trauma when inserted into the brain and potentially affect the pO_2 measurements (3). Optical techniques have a very limited depth of measurement (< 2 mm) when made non-invasively from the surface. An alternate approach using an insertable fiberoptic probe can provide measurements at greater depths, but cause acute trauma at the time of measurement (6,9). On the other hand, the methods based on hemoglobin do not provide direct measure of tissue pO_2 . MRI, PET and EPR imaging require repeated injections of contrast agents, tracers or oximetry probes for serial measurement of the oxygen levels in the tissue of interest (4,7,8). EPR imaging with water soluble probes provide either an average pO_2 over a large tissue volume (~ 0.5 cm³) with high temporal resolution using conventional spectroscopy or high spatial resolution (~ 1 mm³) though imaging (5). These methods have provided very useful information, however, the need for repeated injection of the probes and tissue trauma associated with some methods limits in vivo applications.

We have focused on the development of EPR oximetry using particulate probes, which requires a one-time implantation of the probe by a minimally invasive procedure but rest of the method for pO_2 measurement is entirely non-invasive and can be repeated as desired (2,10–12). The basis of EPR oximetry is the paramagnetic nature of molecular oxygen, which affects the EPR line widths of other paramagnetic species, such as LiPc (oximetry probe), in its vicinity by altering their relaxation rates. The magnitude of change in the EPR line width is directly related to the amount of oxygen that is present in the environment of the paramagnetic probes. The useful properties of LiPc for EPR oximetry are their stability and the strong response of their spectra to the presence of oxygen. The physicochemical properties of the LiPc crystals have been described earlier (1,13)

Tissue pO_2 measurements using 1.2 GHz EPR spectrometers can be made at depths of less than 10 mm from the surface, which has limited its application to mainly small experimental models. Consequently, we have developed IRs that can be used to assess pO_2 at depths greater than 10 mm from the surface (14). We report the application of IRs with four-probes to assess the dynamic changes in the regional oxygen levels at different depths (3 mm and 5 mm) during ischemia by MCAO in rats. We also have investigated the effect of 100% O_2 breathing on ischemic and contralateral brain pO_2 and its consequence on infarct volume. These results indicate that EPR oximetry with multiple probes IRs can provide crucial information on the temporal changes in the rat brain pO_2 during stroke. We anticipate that this oximetry approach can also be used to study the effect of other pathologies, such as

traumatic brain injury and intracranial hemorrhage, on cerebral pO₂ and develop interventions that can improve oxygen levels to minimize tissue damage.

MATERIALS AND METHODS

Animal and experimental groups

The Dartmouth College Animal Care and Use Program approved the animal protocol. Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA), each weighing 250–300 g were randomly divided into two experimental groups: (i) Control group (30% O₂, N=8); (ii) Hyperoxia group (100% O₂, N=6). All animals had EPR probes placed 5–7 days prior to MCAO. The animals were subjected to a 2 h of ischemia followed by reperfusion and sacrifice 22 h later.

Transient middle cerebral artery occlusion (MCAO)

Animals were anesthetized with 2–2.5% isoflurane in 30% O₂ for all surgical procedures. During surgery, the body temperature was monitored by a rectal probe and the body temperature was maintained at 37 ± 0.5 °C with the help of a warm water pad. The left middle cerebral artery (MCA) was occluded for 2h with subsequent reperfusion according to the method reported by Longa et al. (15) with few modifications (11,12). Briefly, the left common carotid artery (CCA), internal carotid artery (ICA) and external carotid artery (ECA) were exposed through a midline incision of the neck. A silicone rubber-coated monofilament nylon suture (tip diameter 0.26 mm) was inserted into the ECA through a stump, and the CCA was kept open and intact. The suture was advanced into the ICA 18–20 mm beyond the carotid bifurcation for occlusion to induce ischemia. The neck incision was closed with a silk suture and the rats were kept anesthetized. The incision was re-opened 2h later and the intraluminal suture was carefully withdrawn to allow reperfusion. The CCA and ICA were inspected to ensure the return of pulsation indicative of reperfusion. The incision was again closed and the animals were allowed to recover from anesthesia. The rats then were kept for 22 h with free access to food and water.

All rats were sacrificed after 22 h of reperfusion and the brains were carefully removed and sectioned into 2 mm thick coronal sections, starting 2 mm from the frontal pole. The slices were placed in a 2% 2',3',5'-triphenyl-2H-tetrazolium chloride (TTC) solution at room temperature to stain viable brain, which demonstrates mitochondrial dehydrogenase activity (16). The sections were photographed and the volume of the infarct was determined by an investigator blinded to the groups.

Paramagnetic material and implantable resonators (IRs)

The oxygen sensitive LiPc crystals were synthesized in our laboratory (13). The IRs were constructed with a copper wire of diameter 0.15 mm and had two sets of loops, a larger loop (coupling loop) on one end and four small loops (sensor loops (SLs)) on the other end of the transmission line (T), Figure 1. The coupling loop has a diameter of 10 mm and is used to couple inductively to the surface loop resonator of the L-band EPR spectrometer. The sensor loops (inner diameter of 0.2 – 0.3 mm) were loaded with LiPc crystals (50 – 80 µg) and the entire IR was coated by gas permeable Teflon AF2400 (14,17,18). The SLs were implanted

in the site of interest, and the coupling loop was placed on the skull below the skin for coupling with the surface loop resonator of the EPR spectrometer (Figure 1a and 1b). The length of the transmission lines (i.e. depth of tissue probed) was 3 mm for SL1, and SL4 and 5 mm for SL2 and SL3 from the surface. The SL1 and SL2 were located in the normal cortex and striatum of the contralateral hemisphere whereas the SL 3 and SL4 were located in the striatum (peripheral area of the infarct) and cortex (core of the infarct) during MCAO, respectively. The IRs were calibrated before implantation into the brain (Figure 1c) and the EPR spectra reflect the average pO_2 on the surface of each SL $\sim 0.11\text{--}0.15\text{ mm}^2$. The diameter and the number of sensor loops, the length of the transmissions lines and the distance between sensor loops can be adjusted based on the experimental requirements.

Multi-site EPR oximetry with IRs

For implantation of IRs, the rats were anesthetized (2–2.5% isoflurane in 30% O_2) and a small incision (1.5–2.0 cm) was made on the skin and four burr holes were drilled by using a 23 gauge needle on the skull at predefined co-ordinates (2.5 and 4 mm left and 2.5 and 4 mm right of the midline and 1 mm posterior to the bregma). Four probes of IRs were gently inserted into the brain tissue through these burr holes by using a stereotaxic frame. The holes were cleaned and sealed with bone wax, and the skin sutured.

The oximetry measurements were performed on a 1.2 GHz EPR spectrometer equipped with a surface loop resonator specially designed for in vivo experiments (19,20). The rats were placed between the poles and the head positioned in the center of the EPR magnet. The surface loop resonator was gently placed over the head region with the coupling loop of IRs and adjusted to obtain the best tuning of the spectrometer. A magnetic field gradient of 3.0 G/cm was used to separate the EPR spectra from each SL (21). The peak-to-peak line widths of the EPR spectra were used to determine pO_2 by using appropriate calibration of the IRs used in the study.

Experimental protocol

In vitro phantom experiments—The in vitro experiments were carried out in 2.0% agarose gel (phantom) to compare the EPR line width (LW) and S/N of the IRs with different lengths of the transmission lines: (i) mm and 11 mm (IR-T6 & 11), and (ii) 3 mm and 5 mm (IR-T3 & 5) (Figure 2a & 2b). Four aggregates of LiPc crystals (approximately 100 μg each) were directly implanted using a 23 Ga needle/plunger into the gel at the same depth (Figure 2c). Three to four samples for each experiment were used to compare the LW and S/N of IRs with LiPc deposits. The EPR settings for in vitro experiments was: the incident microwave power: 0.08–0.8 mW for IRs, 0.8–80 mW for LiPc deposits; gradient: 2.8 – 70 mT/m for IRs and LiPc deposits; modulation frequency 24 kHz; magnetic field center 425 G; scan time 10 sec, scan range 3 – 30 G, modulation amplitude not exceeding one third of the peak-to-peak line width.

In vivo experiments—Five to seven days after the implantation of IRs with transmission lines of 3 mm and 5 mm (T 3 & 5 mm), the animals were anesthetized (1.5% isoflurane with 30% oxygen) and a pre-treatment (baseline) pO_2 measurements was made for 30 min at the four sites. Thereafter, MCAO was performed and pO_2 measurements were repeated for 30

min during which all the animals continued to breathe 30% oxygen. After the confirmation of a significant decrease in pO_2 (ischemia), the breathing gas was switched to 100% O_2 for 60 min and then switched back to 30% O_2 in the last 30 min of MCAO. The animals of the control group continued to breathe 30% O_2 during MCAO. The typical EPR spectra collected during the baseline, ischemia and reperfusion are shown in Figure 3. During the experiments, the body temperature of the rats was maintained at $37 \pm 0.5^\circ\text{C}$ using a heated water blanket and warm air forced through the core of the magnet. The heart rate (H) and blood hemoglobin oxygen saturation (SpO_2) were continuously measured by a Pulse Oximeter (Nonin 8600V Pulse Oximeter, Phoenix, Arizona) throughout the experiments. The EPR settings for in vivo experiments were: the incident microwave power: 0.08–0.8 mW, gradient: 70–168 mT/m and other parameters described for in vitro experiments.

Statistical analysis

A paired T test was used to compare the pO_2 values at four sites (SL) within the same group. The paired comparison reduces the effects of animal-to-animal heterogeneity and eliminates differences in the baseline pO_2 . The comparisons between groups including line width and S/N ratio, the pO_2 values and infarct volume were made using a student's T test for unpaired samples. The tests were two-sided and a change with a p-value <0.05 was considered statistically significant. All data are expressed as mean $\pm$ SE, n is the number of in vitro samples and N is the number of rats in each group.

RESULTS

In vitro calibration of IR in gas phase

The line widths of the EPR spectrum acquired from each sensor loop of the IR-T3 & 5 were a linear function of pO_2 , Figure 1c. Some differences in the response of the sensor loops at high oxygen concentrations were observed. Therefore, each sensor loop was calibrated individually and the line width was converted to pO_2 using the corresponding calibration. Similar procedures were followed with the calibration of IR-T6 & 11.

In vitro EPR measurements in phantom (2.0% agarose gel)

The line widths of each sensor loop of IR-T6 & 11 and IR-T3 & 5 implanted in phantom are shown in Figure 4a. No significant differences in the line widths of the sensor loops were observed. The line width of the sensor loops were pooled together and compared with the line width obtained without gradient, Figure 4b. The differences in the LW of the IR-T6 & 11 and IR-T3 & 5 with or without gradient were $4.1 \pm 2\%$ and $2.1 \pm 1.5\%$, respectively. The LW of the IRs recorded without gradient were similar to that of the LiPc deposits, however, the S/N ratio of the LiPc deposits were significantly smaller ($p < 0.01$, Figure 4c, 4d). We were not able to separate the EPR signals from the four LiPc deposits even at higher gradients and microwave power.

Measurement of cerebral pO_2 during MCAO and the effect of 100% O_2

In the control group, no significant difference in the baseline (pre-ischemic) pO_2 between all the sites was observed, Figure 5. The mean pO_2 decreased significantly in the cortex (ischemic core, SL4) during the first 30 min of MCAO ($p < 0.01$), while the pO_2 of the

contralateral hemisphere (SL2 and SL1) remained at the baseline level. The extent of decline in the pO_2 of the core and striatum was $41\pm4\%$ and $84\pm5\%$ of the baseline in the first 30 min of MCAO, respectively (Table 1). There were significant differences in the extent of decline in the pO_2 between the ischemic core and peripheral area during MCAO ($p<0.01$). The mean pO_2 of the core and peripheral area did not return to baseline during the first 30 min of reperfusion ($p < 0.05$). However, the mean brain pO_2 of all the four sites returned to baseline at 22 h of reperfusion (Figure 5, Table 1).

In the 100% O_2 group, no significant difference in the mean baseline pO_2 between the four sites was observed, Figure 6. The extent of decline in the pO_2 in the core and peripheral area during the first 30 min of ischemia was $37\pm4\%$ ($p<0.01$) and $75\pm12\%$ respectively, while the pO_2 of the contralateral hemisphere (SL2 and SL1) remained unchanged. Furthermore, there were significant differences between the ischemic cortex and striatum tissue pO_2 during 120 min of MCAO ($p<0.05$), Fig 6. The pO_2 of the peripheral area was not significantly different from the baseline during 60 min of breathing 100% O_2 , which is different from the control animals. However, the pO_2 declined significantly when the breathing gas was switched back to 30% during the last 30 min of ischemia ($p<0.05$, Table 1 and Figure 6). The pO_2 of the contralateral sites also increased significantly during breathing of 100% O_2 but returned to baseline when the 100% O_2 was discontinued. The pO_2 of the ischemic core and peripheral area remained significantly lower than the baseline only during the first 30 min of reperfusion.

Measurement of animal physiology and infarct volume

Table 2 shows the arterial oxyhemoglobin saturation (SpO_2), heart rate (HR) and core temperature of animals breathing 30 % O_2 or 100% O_2 . SpO_2 values significantly increased after 100% O_2 as compared to the baseline and control group. On the other hand, a significant increase in the H and body temperature during ischemia as compared to the baseline was observed only in the control group.

120min of MCAO followed by 22h of reperfusion resulted in a significant infarct in the ischemic hemisphere of all surviving rats in both groups. However, no significant difference in the infarct volume with 100% O_2 ($200.7 \pm 38.5 \text{ mm}^3$) compared to the control group ($251.8 \pm 37.1 \text{ mm}^3$) was observed. These results are consistent with our previous finding (12).

DISCUSSION

We have developed IRs to overcome the limited depth of tissue pO_2 measurement with direct implantation of particulate oximetry probes at L-band EPR (14,17,22). The IRs were constructed from enameled copper wires and can be used to measure pO_2 at any depth from the surface. For simultaneous measurement of tissue pO_2 at multiple sites, IRs with multiple SLs can be used with magnetic field gradients. Our in vitro experiments demonstrate a significantly greater S/N with IRs compared to direct LiPc implants. Additionally, the line widths of the IRs with different lengths of the transmission lines for pO_2 measurement at different depths (IR-T6 & 11 and IR-T3 & 5) had similar line widths in the presence or absence of the magnetic field gradients. These results indicate that the spatial resolution as

small as 1.5 mm between the SLs can be easily achieved by applying appropriate magnetic field gradient without any distortion of the EPR signals.

The pO_2 values at the four sites in the brain were stable over 30 min of baseline measurement in both control and 100% O_2 groups. The tissue pO_2 decreased significantly in the core (SL 4) and peripheral area (SL 3) but the pO_2 of the contralateral hemisphere (SL 2 and SL 1) did not change during MCAO. A significant increase in the pO_2 of the contralateral brain during 60 min of breathing 100% O_2 was evident, but no such changes were observed in the ischemic region. Additionally, no significant difference in the infarct volume between the control and 100% O_2 group was observed. These results are consistent with our previous report in which LiPc implants were used for pO_2 measurement in rats subjected to 120 min of MCAO (12). Liu et al have reported the pO_2 of the penumbra using LiPc implants in rats breathing different concentration of oxygen (30%, 70%, 95% or 100%) during 90 min of MCAO (23). The rats breathing 95% or 100% oxygen not only had reduced infarct volume, but also maintained the pO_2 at the pre-treatment level. Singhal et al. noted that 100% O_2 leads to reduced infarct size, measured by serial MRI after 2 h MCAO followed by 1h of reperfusion (24). Pan et al. compared rats breathing mixed oxygen/nitrogen (30%/70%), 100% oxygen, or mixed oxygen/heliox (30%/70%) during filament-induced 120 min of MCAO. A significant decrease in infarct volume (TTC at 24 h) was observed in rats breathing 30%/70% O_2 using heliox (4%) compared to 100% O_2 (16%) (25). However, Beynon et al. did not find any beneficial effect of 120 min of breathing 100% O_2 initiated 60 min after transient filament-induced MCAO for 150 min (26). No significant reduction in the infarct volume or clinical impairment in the 100% O_2 group compared to the control group was observed after 90 min of MCAO by Eschenfelder et al. (27).

No significant increase in the pO_2 of the ischemic regions was observed during 60 min of breathing 100% O_2 in our experiments, which is consistent with no change in the infarct volume compared to the control. The contradicting results discussed above are likely due to a difference in the MCAO model, duration of ischemia, lack of direct measurement of pO_2 to verify the changes in oxygen levels, and different durations of breathing 100% O_2 . Additional studies are planned to systematically investigate the effect of these variables on the cerebral pO_2 , cerebral blood flow and infarct volume.

CONCLUSIONS

These results demonstrate that EPR oximetry using multi-probe IRs potentially can be used to investigate the effect of ischemia-reperfusion on the cerebral pO_2 at several sites simultaneously and repeatedly over days. The IRs provide significantly better S/N ratio as compared to direct LiPc implants and can be used to measure pO_2 at depths greater than 10 mm from the surface. No significant change in the tissue pO_2 of the ischemic region and consequently infarct volume was observed with 60 min of breathing 100% O_2 during 120 min of MCAO as compared to the control group. The IRs based EPR oximetry approach will be especially useful to study cerebral tissue pO_2 in large experimental models, including primates, to develop and test interventions to increase oxygen levels in the ischemic regions to salvage cerebral tissue in stroke.

Acknowledgments

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Figure 1.

a: Axial view of rat skull with trephination positions of the sensor loops. **b:** Schematic of rat brain showing the location of the infarct in the brain (shaded area) and the location of the sensor loops in the ischemic core (sensor loop 4, SL4), peripheral area (sensor loop 3, SL3) and contralateral striatum (sensor loop 2, SL2), and cortex (sensor loop 1, SL1). AP: anterior-posterior; ML; medial-lateral; DV: dorsal-ventral. **c:** Calibration plot of a 4-SL IR. The response of each SL to different concentrations of perfused oxygen and regression coefficients (R^2).

Figure 2. Schematic of phantoms (2.0% agarose gel) used for in vitro experiments with an IR fabricated with (a) 6 mm and 11 mm transmission lines, IR-T6 & 11, (b) 3 mm and 5 mm transmission lines, IR-T3 & 5 and (c) LiPc deposits at depths of 3mm and 5 mm. Typical EPR spectra recorded from (d) IR-T6 & 11, (e) IR-T3 & 5 and (f) LiPc deposits.

Figure 3.

The typical EPR spectra from an implanted multiple SL-IR (IR-T3 & 5) for the simultaneous measurement of cerebral pO₂ in both hemispheres of a rat brain recorded at 0 min (baseline), 30, 60, 90 min of ischemia (I), 30 min of reperfusion (R), and 22 h of reperfusion (R/22 h). The narrowing of the line width (intense EPR lines) corresponds to a decrease in local pO₂ when compared with the baseline. The numbers shown are the pO₂ values in mmHg acquired from each SL using multi-site EPR oximetry.

Figure 4.

Line width (LW) and signal to noise (S/N) ratio of IR-T6 & 11, IR-T3 & 5 and LiPc deposits in phantoms (2.0% agarose gel). **a**, the LW acquired from each SL of IR-T6 & 11 (grey column) and IR-T3 & 5 (black column); **b**, the LW of the four SLs with gradient applied (solid column) and the LW obtained without applied gradient (upward diagonal) from the IR-T6 & 11 and IR-T3 & 5; **c & d**, LW and S/N of the IR-T6 & 11, IR-T3 & 5 and LiPc deposits without applied gradient. Mean $\pm$ SE, $n = 3 - 5$. ** $p < 0.01$, compared with IR-T6 & 11 and IR-T3 & 5, respectively.

Figure 5.

Dynamic changes of mean cerebral pO₂ in the control group in ischemic cortex (□), striatum (●) and contralateral striatum (○) and contralateral cortex (■) before, during and after middle cerebral artery (MCA) occlusion in rats (mean ± SE, N = 8). BL: baseline; I: ischemia; R: reperfusion; R/22 h: reperfusion at 22 h. † p < 0.05, †† p < 0.01, compared with baseline pO₂ in same site; ‡‡ p < 0.01, compared with site 3 (ischemic striatum, SL3).

Figure 6.

Dynamic changes of mean cerebral pO₂ in 100% O₂ group in the ischemic cortex (□), striatum areas (●) and contralateral striatum (○) and contralateral cortex (■) before, during and after middle cerebral artery (MCA) occlusion in rats (mean ± SE, N = 6). Shaded area indicates the duration of the 100% O₂ inhalation. BL: baseline; I: ischemia; R: reperfusion; R/22 h: reperfusion at 22 h. †p < 0.05, ††p < 0.01, compared with baseline pO₂ in same site; ‡p < 0.05, ‡‡p < 0.01, compared with site 3 (ischemic striatum, SL3).

percentage changes of baseline cerebral pO₂ during 2 h of ischemia, during the first 30 min of reperfusion and after 22 h of reperfusion

Table 1

Groups	Sites	BL (mmHg)		I (% of BL)				R (% of BL)		R/22 h (% of BL)
		0-30 (min)		0-30	31-60	61-90	91-120	0-30 (min)		
				(min)						
100% O₂ (N=6)	1	51±2.7	102±7	141±15 [†]	145±12 [†]	111±9	99±4	99±4		90±3
	2	50±2.3	96±3	146±13 [†]	155±9 ^{††}	109±5	95±5	95±5		89±5
	3	50±5.9	75±12	93±18	95±24	67±10 [†]	76±10	76±10		91±17
	4	48±3.4	37±3 ^{††}	47±5 ^{†††}	41±5 ^{†††}	31±5 ^{††††}	67±11 [†]	67±11 [†]		88±10
Control (N=8)	1	55±2.1	100±6	96±7	91±7	87±6	92±8	92±8		96±4
	2	51±3.6	110±11	105±10	98±9	97±11	103±11	103±11		81±3 [†]
	3	53±5.4	84±5 [†]	73±4 [†]	68±4 [†]	66±4 [†]	68±9 [†]	68±9 [†]		99±15
	4	53±3.7	41±4 ^{†††}	36±3 ^{††††}	33±3 ^{††††}	29±3 ^{††††}	66±9 [†]	66±9 [†]		98±10

BL: baseline; I: ischemia; R: reperfusion; R/22h: reperfusion at 22 h. Values are mean ± SE.

[†] p < 0.05,

^{††} p < 0.01, compared with baseline pO₂ in same site;

[‡] p < 0.05,

^{‡‡} p < 0.01, compared with site 3 (ischemic striatum, SL3).

Table 2

Physiological parameters

Group	Parameters	BL (min)		I (min)		R (min)	
		0-30	31-90	0-30	91-120	0-30	R/22h (min)
100% O₂ (N=6)	SpO ₂ (%)	92±2.1	90±2.0	97±0.7 ^{†‡}	91±1.3	92±1.2	91±3.2
	HR (beats/min)	355±7	360±20	376±17	389±19	77±15	350±11
	Temp. (°C)	37±0.1	36±0.2	37±0.3	37±0.4	37±0.2	36±0.3
Control (N=8)	SpO ₂ (%)	93±1.5	93±1.8	94±1.2	94±0.7	94±1.7	95±1.1
	HR (beats/min)	388±20	401±29	443±21 ^{†‡}	458±18 ^{†‡}	412±16	383±21
	Temp. (°C)	36±0.2	36±0.4	38±0.3	38±0.3 [†]	37±0.2	37±0.1

BL: baseline; I: ischemia; R: reperfusion; R/22h: reperfusion at 22 h; SpO₂: arterial oxyhemoglobin saturation; HR: heart beat rate; Temp.: core body temperature. Values are mean ± SE.

[†] p<0.05,

^{†‡} p<0.01, compared with baseline;

[‡] p<0.05, compared with control group.