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Control of brain temperature during experimental global ischemia in rats

Ansgar M. Brambrink [a,b,*], Laszlo Kopacz [a], Andreas Astheimer [a,b], Holger Noga [a,b], Axel Heimann [a], Oliver Kempski [a]

[a] *Institute for Neurosurgical Pathophysiology, Johannes Gutenberg-University, Langenbeckstraße 1, D-55101 Mainz, Germany*
[b] *Department of Anesthesiology, Johannes Gutenberg-University, Langenbeckstraße 1, D-55101 Mainz, Germany*

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Abstract

Temperature control during experimental ischemia continues to be of major interest. However, if exposure of brain tissue is necessary during the experiment, regional heat loss may occur even when the core temperature is maintained. Furthermore, valid non-invasive brain temperature monitoring is difficult in small rodents. This paper describes a method for both monitoring and maintenance of brain temperature during small animal preparations in a stereotaxic frame. The device used includes an ear-bar thermocouple probe and a small near-infrared radiator. The new equipment permitted to maintain peri-ischemic brain temperature at a desired level while carrying out non-invasive continuous recordings of cerebral blood flow (laser Doppler-flowmetry) and of electrical brain function (EEG). In contrast, without extracranial heat application, superficial and basal brain temperatures decreased during global cerebral ischemia by 4.1 ± 0.1 and 4.6 ± 0.4°C (mean ± SEM), respectively, returning to baseline values at 15–30 min of reperfusion while rectal (core) temperature remained stable at baseline values. The ear-bar thermocouple probe (tympanic membrane) reliably reflected basal brain temperature, and temperature in superficial brain areas correlated well with that in the temporal muscle. Our data show that the new system allows to exclude unwanted hypothermic neuroprotection, and does not interfere with optical and electrical measurement techniques. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Brain temperature; Ear-bar thermocouple probe; Hypothermia; Ischemia; Neuroprotection; Near-infrared radiator; Rat; Tympanic membrane

1. Introduction

Brain temperature determines neuronal injury and long-term outcome resulting from global cerebral ischemia (for review: Dietrich, 1992; Ginsberg et al., 1992; Clifton, 1995; Marion et al., 1996; Wass and Lanier, 1996; Colbourne et al., 1997).

The substantial influence of reduced temperature during ischemia on long-term outcome is well-known. Intra-ischemic reduction of temperature protects the brain from post-ischemic edema (Schwab et al., 1998), diminishes neuronal injury (Dietrich et al., 1990a,b, 1993; Freund et al., 1990; Minamisawa et al., 1990a,b; Welsh et al., 1990; Horn et al., 1991; Laptook et al., 1995; Lin et al., 1995; Corbett et al., 1997; Williams et al., 1997), and improves functional (Burger et al., 1998) and behavioral outcome (Green et al., 1992; Laptook et al., 1994; Nathan et al., 1995; Bona et al., 1998). In contrast, elevation of temperature is associated with an accelerated (Dietrich et al., 1990b; Ginsberg and Busto, 1998) and more extensive injury (Dietrich et al., 1990b, 1991; Minamisawa et al., 1990b; Azzimondi et al., 1995; Globus et al., 1995).

However, there is a surprising lack of detailed reports on achieving protection against unwanted hypothermia during various protocols of experimental ischemia, i.e. on appropriate monitoring and meticulous active adjustment of brain temperature before, during and after the cerebral insult. Measurements of body temperature,

* Corresponding author. Tel.: +49-6131-172373; fax: +49-6131-176640.

E-mail addresses: brambrin@mail.uni-mainz.de (A.M. Brambrink), kempski@nc-patho.klinik.uni-mainz.de (O. Kempski)

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e.g. by rectal probe, do not reflect brain temperature during and after an ischemic insult (Busto et al., 1987). A thermocouple probe placed in the temporal muscle may not show the actual temperature of deep brain areas: (1) because of its superficial extracranial position; and (2), if an additional heat source is placed above the head, the measurement may be adversely affected. In contrast, tympanic membrane temperature reflects epidural, i.e. whole brain temperature very closely (Mellergård and Nordström, 1990; Mariak et al., 1994). However, conventional tympanal measurements cannot be obtained in small rodents requiring a stereotaxic apparatus for neurosurgical interventions and invasive monitoring.

To measure functional parameters, exposure of the calvarium or of the dura are required as, e.g. for procedures such as laser Doppler-flowmetry [LDF], tissue oxymetry, electroencephalography [EEG], or somatosensory-evoked potentials [SEP]. This exposure renders the brain more susceptible to continuous temperature loss, and when a number of monitoring systems are applied in parallel, maintenance of cerebral temperature at physiologic levels becomes difficult. Conventional methods, such as thermostatically-regulated heating blankets to control whole body temperature do not necessarily prevent a temperature decrease in the brain during ischemia (Busto et al., 1987). The use of a conventional heating lamp, placed above the head (Busto et al., 1987; Minamisawa et al., 1990c) may significantly interfere with the wavelength of optical instruments, e.g. LDF or tissue oxymetry. This is of particular importance when regional cerebral blood flow [rCBF] needs to be monitored with a 'scanning technique' (Heimann et al., 1994; Kempski et al., 1995; Nakase et al., 1997) requiring several minutes per scan, or when continuous measurements are necessary to achieve a high temporal resolution of cerebral blood flow changes. In addition, superfused warm saline solution (Colbourne et al., 1993) makes parallel electrophysiological measurements (EEG, SEP) impossible.

The purpose of this study was therefore to develop a system for peri-ischemic temperature control in small rodents for application in combination with various optical and electrical measurement techniques, and to investigate the benefit of this system in a model of global cerebral ischemia in rats.

2. Materials and methods

2.1. Ear-bar thermocouple probe to measure tympanal temperature

A thermistor probe (EDSLAB® T.D. Probe, Model 94-030-2.5F, originally designed for invasive blood temperature measurements) was introduced into the tip of a stereotaxic pin to allow temperature measurements at the tympanic membrane with the animal in a stereotaxic apparatus (Fig. 1).

We modified a stereotaxic ear-bar by cutting off the tip and replacing it with a thermistor tip embedded in polyacethal resin to provide insulation from the surrounding metal. The thermistor can be connected to the Oximetrics 3 (Abbott, North Chicago, IL, USA) or other temperature monitors.

2.2. Near-infrared heat radiator to control peri-ischemic brain temperature

We developed a near-infrared radiator designed to distribute energy down to basal areas of the rat brain without overheating superficial brain areas. The spectrum of the radiator was set to avoid any interference with LDF. The instrument is so small as to fit into the limited space of experimental settings which include a small animal stereotaxic apparatus (Fig. 1).

The device consists of a heating element (aluminum oxide [AlO_2], manganese nickel-chromium [MnNiCr], black surface, 10.9×3.7 mm, total emissivity (ε) = 0.89–0.97 at 500°C) placed at the center of an ellipsoid reflector (99% aluminum, polished, 21×28 mm, reflection index [R] = 89.9–99.4%). The heating element is regulated by a direct current [DC]-transformer (dual-output power supply EA-3023, Elektro-Automatik, Viersen, Germany) to a temperature of up to 500°C (4 volt [V], 1–1.25 ampere [A], resulting in maximal 6 watt [W]) leading to the emission of near-infrared radiation (wavelength [λ] = 800 nm to $>$ 10 μm) with a peak λ at 3.5 μm. The spectrum of electromagnetic irradiation was determined on the basis of spectral radiant emittance at 800 K (Moore, 1986).

In our experiment, the near-infrared heat radiator was placed 4 cm above the calvarium, resulting in a heat spot with a 10 mm radius covering the entire exposed area of the skull. In this setting an area of at least 2 cm in diameter was thus homogeneously irradiated, with additional engergy being diffused beyond this area. The DC-transformer was regulated between 4 and 6 W, to ensure a basal brain temperature of 37.5 ± 0.1°C without increasing superficial brain temperature above that level.

2.3. Animal preparation

All experimental procedures were approved by the regional ethics committee for animal research (Regional Government Rheinhessen-Pfalz).

Wistar rats ($n = 11$, Charles River, Kißlegg, Germany) weighing 309 ± 19 g (mean $\pm$ SEM) were used in this study. The animals had free access to food and water. They were anesthetized using chloral hydrate (induction: 360 mg/kg body weight intraperitoneal

[i.p.]; maintenance: 120 mg/kg per h, i.p.) and received atropine (0.5 mg, subcutaneous [s.c.]). Tracheal intubation was then carried out and the animals were mechanically ventilated (small animal ventilator, AP-10, Effenberger, Pfaffing/Attel, Germany) using a fraction of inspired oxygen [FiO_2] of 0.30 in humidified air. Initially, ventilation was set to control end-tidal carbon dioxide ($EtpCO_2$) at around 40 ± 2 mmHg (Capnomac ultima, Datex Engstrom, Helsinki, Finland), later with vascular access to control arterial carbon dioxide pressure [$PaCO_2$] at 35–40 mmHg. Arterial oxygen pressure [PaO_2] of 95–110 mmHg was maintained throughout the experiment (ABL System 615, Radiometer, Copenhagen, Denmark). Both common carotid arteries (CCA) were exposed and the left side was catheterized with polyethylene tubing (PE-50) to allow blood sampling and continuous monitoring of mean arterial blood pressure (MABP, Sirecust 310, Siemens, Danvers, MA, USA). A nylon thread was looped loosely around the CCA on the right side and later used to produce transient cerebral ischemia.

The head of the animal was fixed in a stereotaxic frame for EEG, local cerebral blood flow (lCBF) and temperature measurements. The calvarium was exposed by a median incision and two depressions were made using a high-speed dental drill (Microtron 60, Typ GD 612, Aesculap, Tuttlingen, Germany) to hold pin electrodes for EEG recordings. One pin electrode was placed over the left somatosensory cortex (3.5 mm lateral to the left, over the bregma = recording electrode) and a second electrode was fixed above the frontal sinus (1 mm lateral to the left, and 4 mm rostral from the bregma = reference electrode). Chlorinated silverball electrodes served to record the electrical signal, which was bandpass filtered (5–500 Hz), amplified (20 mV) and displayed using a conventional neurophysiologic monitoring unit (Neuropack II, Nihon Kohden, Tokyo, Japan).

Additionally, a portion of the skull was removed 1.3–5.3 mm lateral to the right and 1.5–7.5 mm occipital from the bregma using the same high-speed dental drill, which resulted in a cranial window of about 24 mm^2 (the dura remained intact). A LDF probe (probe: P 433-2, monitor: BPM 2, Vasamedics, St Paul, MN, USA; wavelength: 780 nm), controlled with a manually driven micro-manipulator was used to determine the lCBF in the center of that field. The probe was positioned to monitor an area of brain microcirculation (lCBF = 20–30 LDF units at baseline), and to avoid small blood vessels.

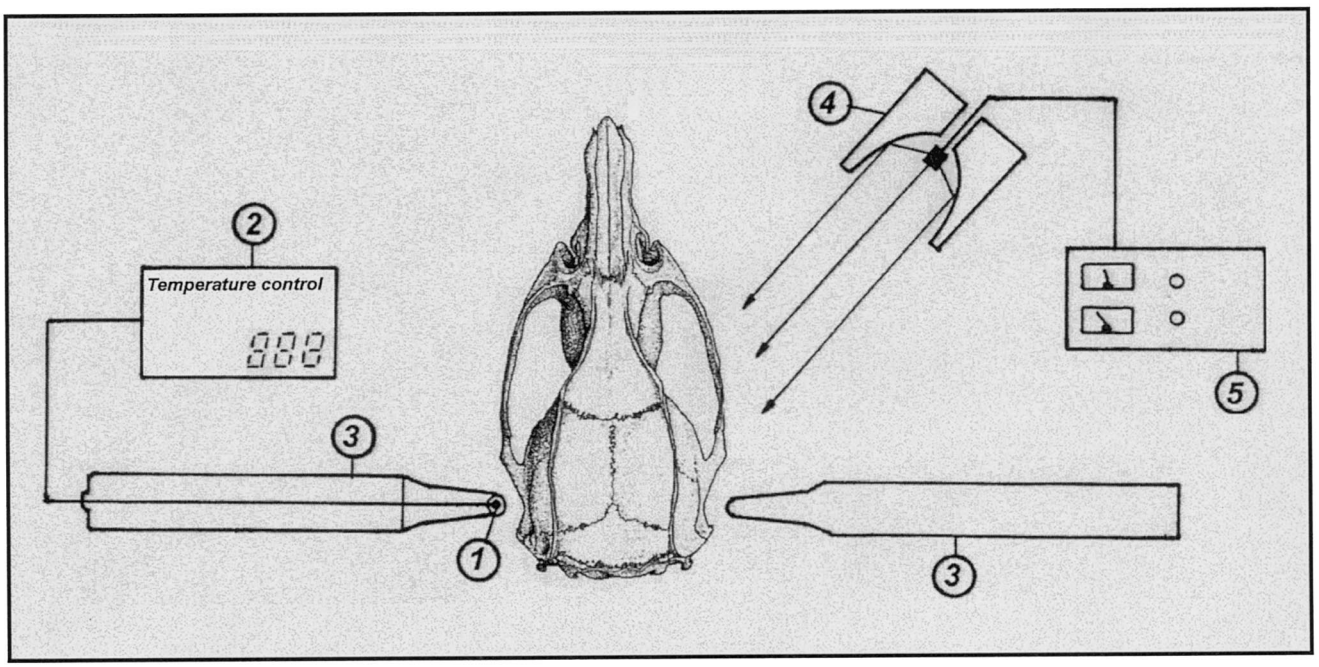

Fig. 1. Setup of the temperature control system for small rodents. The temperature probe for tympanal temperature measurements within the stereotaxic apparatus, consists of a modified stereotaxic ear-bar (3) with a thermistor tip (1) embedded in polyacethal resin for insulation. The ear-bar thermocouple probe is connected to an electronic temperature control monitor (2). The small near-infrared heat radiator (4) consists of a black surface heating element (aluminum oxide, manganese nickel-chromium, 10.9×3.7 mm) placed at the center of an ellipsoid reflector (99% aluminum, polished, 21×28 mm, reflection index [R] = 89.9–99.4%). The heating element is regulated by a direct current transformer (5) to a temperature of up to 500°C, resulting in the emission of near-infrared light ($\lambda = 800$ nm to > 10 μm, peak λ at 3.5 μm). The near-infrared heat radiator is placed 4 cm above the calvarium, producing a 10-mm radius heat spot. The entire exposed area is homogeneously irradiated, with additional energy being diffused beyond this area. The DC-transformer is regulated between 4 and 6 W to ensure a basal brain temperature of 37.5 ± 0.1°C without increasing superficial brain temperature above physiologic levels.

The lower body section of the animal was placed in an air-tight chamber, which allows to produce hypobaric pressures with an electronically-regulated vacuum pump, and thus to reduce arterial blood pressure (venous pooling) in a controlled fashion ('hypobaric hypotension'; Soehle et al., 1998).

2.4. Temperature monitoring during the experiment

Two thermocouple probes (0.25 mm diameter) were placed at designated coordinates within the cranial window to monitor temperature of both the rostral and the occipital hippocampus (Licox pO2 Monitor, GMS, Kiel, Germany). Probe positions were defined according to Paxinos and Watson (1986), the correct placement having been confirmed in pilot experiments. One probe was advanced 2 mm into the rostral hippocampus (3.0 mm lateral and 3.0 mm occipital of the bregma) to measure superficial brain temperature (SBT), and a second to 5 mm depth into the occipital hippocampus (4.5 mm lateral and 6.0 mm occipital of the bregma) to measure basal brain temperature (BBT). Additional temperature probes were established in the right temporal muscle (Oximetrix 3, Abbott, North Chicago, IL, USA), in the left auricular tube (tympanal temperature probe within a stereotaxic pin, self-constructed device, Fig. 1) and in the rectum at 6 cm depth (Miyazawa and Hossmann, 1992). The latter was connected to a thermostatically controlled heating blanket to maintain the core temperature at 37.5 ± 0.1°C (Homeothermic blanket control unit 50-7087, Harvard, Edenbridge, UK). The thermocouple probes were calibrated before every experiment, using a conventional mercury thermometer in water over a range of 30–40°C.

2.5. Global brain ischemia and reperfusion

After a post-surgical stabilization period of 30 min, 15-min transient global cerebral ischemia was produced by pulling the nylon thread with an attached weight to occlude the right carotid artery and by simultaneously reducing MABP to 35 mmHg, using the air-tight chamber. Brain ischemia was confirmed by continuous lCBF measurements. The thread was then cut and the vacuum was eliminated to allow reperfusion.

Temperatures at all sites, lCBF and MABP were continuously monitored and recorded at 30, 22, 15, 13, 7 and 1 min before ischemia, at 2 and 15 min during ischemia, and at 3, 10, 30, 60, 62, 70 and 90 min of reperfusion. Arterial blood gases, pH, base excess, hematocrit, hemoglobin, blood glucose [GLU], and lactate [LAC] levels were determined 7 min before ischemia [baseline] and after 3 and 60 min of reperfusion.

2.6. Experimental design

In the described series of experiments we tested two hypotheses: (1) non-invasive tympanal temperature measurement using the stereotaxic probe represents brain temperature throughout the experiment; (2) using the near-infrared radiator, brain temperature before, during, and after global brain ischemia can be maintained within a designated range, and optical and electrophysiological measurement techniques remain unaffected.

Animals were divided at random into two groups: Group I ($n = 5$) – 15 min of global cerebral ischemia followed by 90 min of reperfusion without extracranial heat application, Group II ($n = 6$) – 15 min global ischemia and 90 min reperfusion with extracranial heat application, using the new heating device with invasively measured basal brain temperature as reference. Additionally, animals in Group II served as their own controls as they were kept without extracranial heat radiation during the first part of the postsurgical stabilization period (before the insult) and during the late period of reperfusion (after the insult).

2.7. Statistical analysis

Data are presented as mean values $\pm$ SEM. Temperature measurements, cerebral blood flow values and physiologic parameters were assessed by Student's t-test or rank sum test if required, using SigmaStat®-2.0 routines (Jandel, Erkrath, Germany). Linear regression analysis was used to calculate the correlation between temporal muscle and superficial brain temperatures, and between tympanic membrane and basal brain temperatures, respectively. Differences were considered as statistically significant at $P < 0.05$.

3. Results

Physiological variables of all animals were within normal ranges at baseline. Immediately after the insult (3 min), the animals presented with mild metabolic acidosis, independently of whether extracranial heat was applied or not. Mild acidosis was still present 1 h later, although serum lactate had already returned to baseline values in both groups (Table 1). EEG was isoelectric in every animal within 1 min of ischemia (lCBF close to zero). All animals except two (one per group) showed robust postischemic reperfusion and simultaneous functional (EEG) recovery of the brain and were included for data evaluation.

Fig. 2 shows a comparison of superficial brain temperature (i.e. rostral hippocampus) and temporal muscle temperature. Before the application of additional extracranial heat, superficial brain temperature (panel

Table 1
Physiologic variables[a]

		Baseline	Reperfusion (3 min)	Reperfusion (60 min)
Arterial pH (units)	Without EHA	7.37 ± 0.01	7.26 ± 0.03	7.33 ± 0.02
	With EHA	7.37 ± 0.01	7.32 ± 0.01	7.3 ± 0.03
Arterial PCO_2 (mmHg)	Without EHA	38.17 ± 1.38	36.67 ± 3.72	32.57 ± 3.16
	With EHA	33.3 ± 2.68	28.32 ± 2.07	30.7 ± 3.39
Arterial BE (units)	Without EHA	−3.1 ± 0.73	−10.61 ± 1.22	−8.19 ± 1.11
	With EHA	−5.39 ± 1.21	−10.53 ± 0.82	−10.29 ± 1.27
Arterial PO_2 (mmHg)	Without EHA	112.71 ± 4.38	134.03 ± 8.97	114.27 ± 4.15
	With EHA	123.34 ± 7.5	144.62 ± 7.02	128.09 ± 11.23
Arterial O_2 saturation (%)	Without EHA	98.34 ± 0.98	98.19 ± 2.11	98.01 ± 0.61
	With EHA	99.6 ± 0.26	100 ± 0	98.6 ± 0.71
Serum glucose (mg/dl)	Without EHA	209.2 ± 12.99	207.33 ± 25.31	154 ± 7.53
	With EHA	157.14 ± 20.15	141.6 ± 30.16	139.14 ± 16.14
Serum lactate (mmol/dl)	Without EHA	0.82 ± 0.06	4 ± 0.8	0.92 ± 0.07
	With EHA	0.97 ± 0.12	3.08 ± 0.39	1.11 ± 0.11

[a] All values are mean ± SEM. No statistically significant differences between groups. EHA, extracranial heat application; BE, base excess.

A) was low, ranging from 33.0 ± 0.2 to 33.8 ± 0.5°C (mean ± SEM) in both groups. Superficial brain temperature decreased further by 4.1 ± 0.1 to 29.5 ± 0.5°C at 15 min ($P < 0.001$) of global ischemia when the animals were without extracranial heat radiation (Group I, $n = 4$). Temperature in superficial parts of the brain slowly increased in these animals during the first few minutes of reperfusion and had returned to near pre-ischemic values (33.2 ± 1.2°C) at 10 min. However, superficial brain temperature remained at a moderately hypothermic level.

In contrast, with the near-infrared radiator (Group II, $n = 5$) superficial brain temperature was elevated to physiologic levels before ischemia (36.3 ± 1.0°C), and was maintained within that range during injury (37.0 ± 0.5°C) and recovery (e.g. at 10 min reperfusion: 36.4 ± 1.0°C). After discontinuation of additional heat (at 60 min of reperfusion) superficial brain temperature in the same animals decreased within 10 min to a level which was similar to both their own baseline values (i.e. before the application of radiation) and that observed in Group I animals (34.7 ± 0.7°C) at the same time point.

Temporal muscle temperature (Fig. 2, panel B) closely reflected superficial brain temperature in both absolute values and time resolution, regardless of whether the animals received additional extracranial heat or not. Analysis of individual temperature measurements revealed a close correlation between temporal muscle and superficial brain temperatures [temporal muscle temperature = 8.197 + (0.760 * rostral hippocampal temperature), $r = 0.85$, $P < 0.001$, Fig. 4, panel A]. In some instances of rapid temperature changes, e.g. with institution of extracranial heat application or reperfusion, the difference between the two measurement sites was larger than 0.5°C.

Fig. 3 shows basal brain temperature (i.e. occipital hippocampus) and parallel measurements of tympanic membrane temperature. Temperature changes in basal areas of the brain showed a similar pattern to that of superficial parts, but ranging at an overall higher temperature level. Without extracranial heat application basal brain temperature was between 36.2 ± 0.6 and 36.9 ± 0.4°C in both groups. When no extra heat was applied (Group I, $n = 4$), basal brain temperature at the end of global ischemia had decreased by about 4.6 ± 0.4 to 31.6 ± 0.7°C, ranging significantly below levels measured before the insult ($P < 0.001$). Temperatures recovered to near baseline levels (35.3 ± 1.1°C) within 10 min of reperfusion.

On additional near-infrared irradiation to the head (Group II, $n = 5$) basal brain temperature was maintained at physiologic levels before, during and after ischemia (37.8 ± 0.1, 36.7 ± 0.3, 37.8 ± 0.2 [10 min]°C, respectively). When radiation was discontinued, temperature in basal brain areas dropped in a similar pattern to that observed for superficial brain temperature. At 70 min of reperfusion, basal brain temperature was comparable to that before the head was irradiated in the same animals (36.9 ± 0.4°C) and to that of Group I animals recorded at the same time.

Using the new ear-bar probe, tympanal temperature correlated well with basal brain temperature [tympanal membrane temperature = 15.305 + (0.559 * occipital hippocampal temperature), $r = 0.77$, $P < 0.001$, Fig. 4, panel B]. However, in some animals with additional heat radiation (Group II) tympanic temperature was lower than basal brain temperature (around 0.5°C) and the differences were greater (up to 1.8°C) during abrupt temperature changes, e.g. with institution of ischemia or reperfusion. Both, the small but systematic underestimation and the delayed equilibration between the two

temperature measurements suggest that the ear-bar probe may not have been in close contact with the tympanic membrane in some animals and that a small interposed air cushion may have been responsible for the observed offset.

Conversely, there was no close correlation between temporal muscle temperature and basal brain temperature or between tympanal temperature and superficial brain temperature, respectively. Differences were > 2.5°C, and appeared to be more pronounced without extracranial heat application.

Table 2 shows the rectal temperatures, which were maintained around 37.5°C before, during and after the insult by means of the thermostatically controlled heating blanket and, except for an unexplained temperature drop at the end of ischemia (15 min), appear not to have been influenced by the additional heat radiation. Differences between rectal temperature and basal or

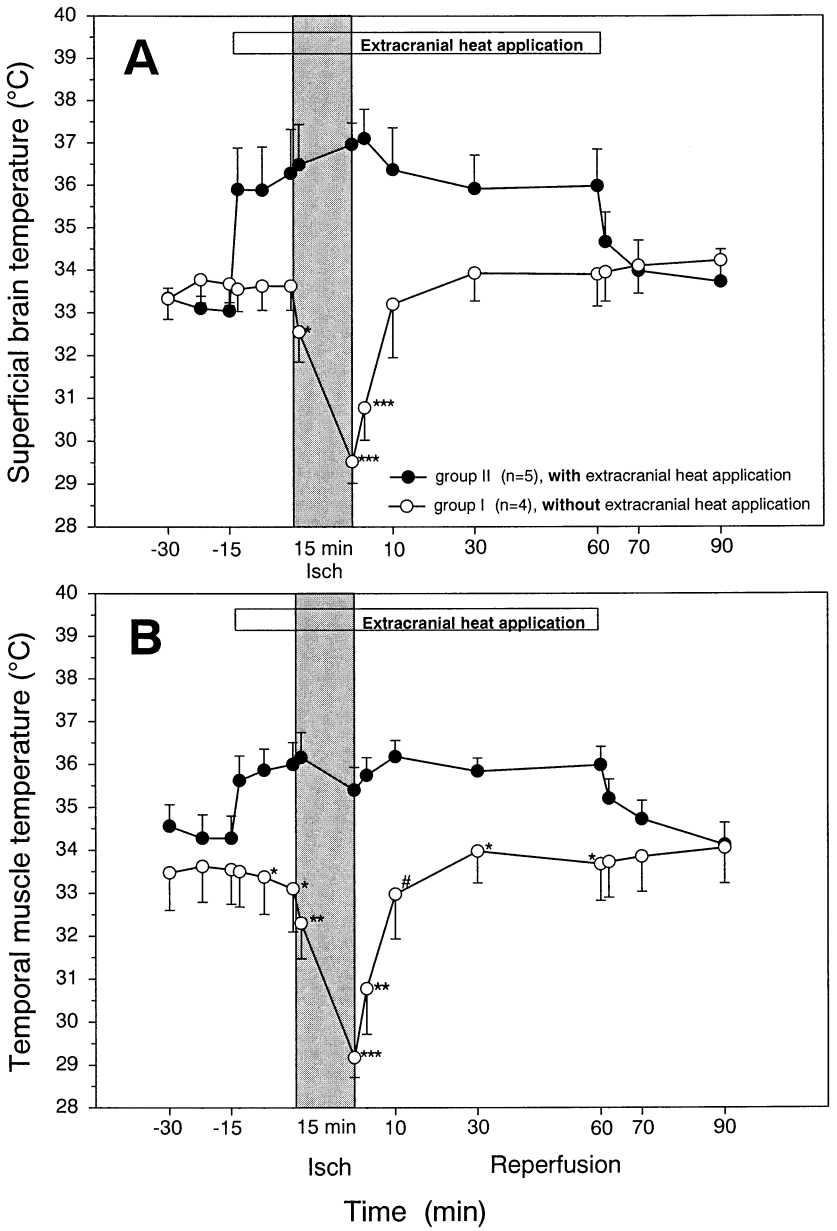

Fig. 2. Superficial (i.e. rostral hippocampal) brain temperature (panel A) and temporal muscle temperature (panel B) are plotted for rats receiving either additional extracranial heat application before, during and after the insult (closed circles, $n = 5$) or no heat radiation (controls, open circles, $n = 4$) during the time window indicated by the bar in the upper section of each panel. Values are means ± SEM recorded at 30, 22, 15, 13, 7 and 1 min before ischemia, at 2 and 15 minutes during ischemia and at 3, 10, 30, 60, 62, 70, and 90 min of reperfusion. With additional extracranial heat both, superficial brain and temporal muscle temperature could be maintained at around 36°C. In the absence of external heat exposure of the head there was a brisk decline in temperatures at both sites during ischemia despite core temperature control. Significant differences between groups are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Student's t-test; $^{\#}P < 0.05$, rank sum test.

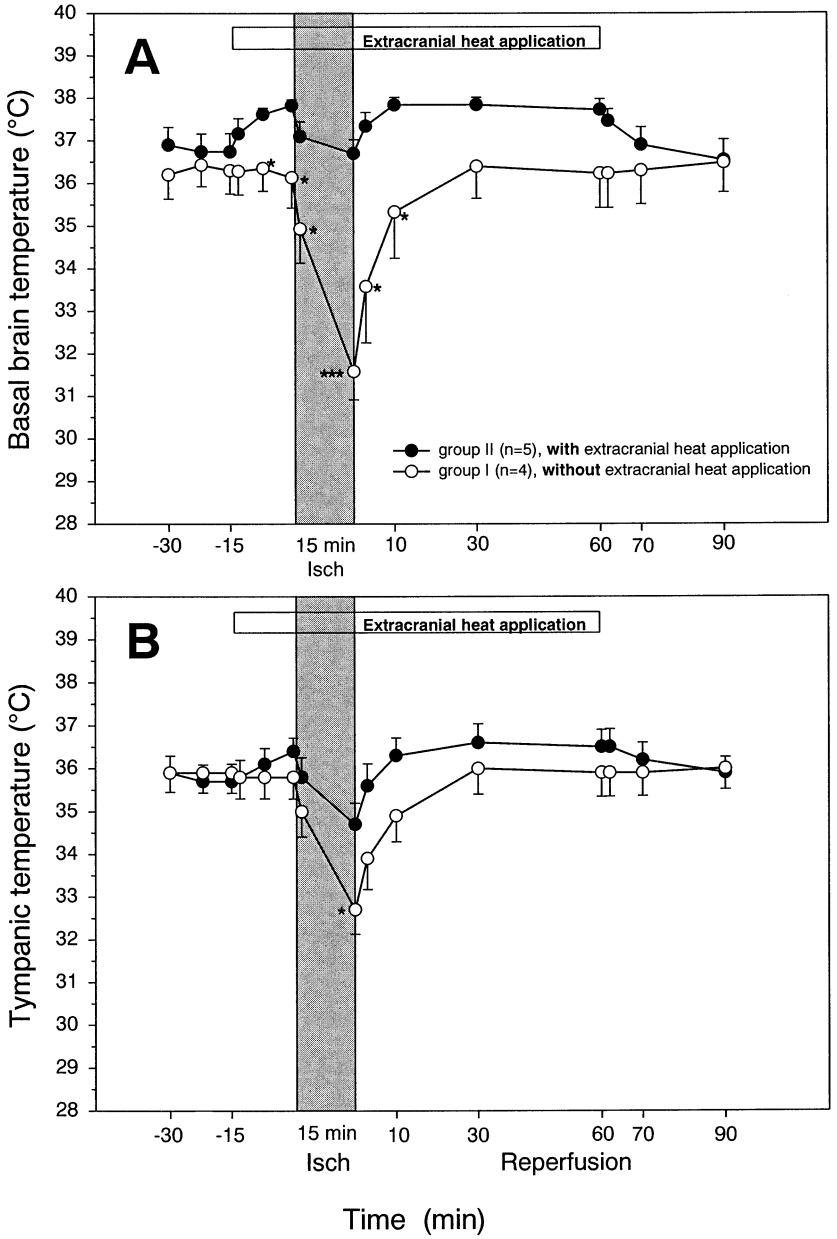

Fig. 3. Temperatures in basal brain areas (i.e. occipital hippocampus, panel A) and tympanic membrane (panel B) are plotted for animals receiving either additional heat application to the head before, during and after global ischemia (closed circles, $n = 5$) or no heat radiation (controls, open circles, $n = 4$). Values are means $\pm$ SEM recorded at 30, 22, 15, 13, 7, and 1 min before ischemia, at 2 and 15 min during ischemia and at 3, 10, 30, 60, 62, 70, and 90 min of reperfusion. Occipital hippocampal temperature was maintained at around 37°C throughout ischemia and reperfusion, using the low-frequency near-infrared heat radiator. In the absence of heat radiation the temperature decreased significantly on both sides during brain ischemia, despite continuous core temperature control with a heating blanket. Basal brain temperature was closely reflected by the new tympanal thermocouple probe. Differences between groups were $^{*}P < 0.05$, $^{***}P < 0.001$, Student's t-test.

superficial brain temperatures were greater without additional heat radiation to the exposed calvarium (e.g. at baseline 1.3 ± 0.7 and 3.8 ± 0.6°C; at 15 min ischemia 5.1 ± 0.4 and 7.1 ± 0.2°C, respectively). In contrast, with the new heat radiator, differences in rectal and basal brain temperature were less marked, i.e. at baseline: 0.4 ± 0.2 and 1.1 ± 1.0°C and at 15 min ischemia 1.6 ± 0.5 and 1.8 ± 0.7°C, respectively.

Table 2 further presents LDF measurements of lCBF and the corresponding MABP throughout the experiment. At pre-ischemic baseline values, MABP and lCBF were at normal levels, independently of whether animals received additional extracranial heat radiation. On initiation of hypobaric hypotension, MABP was reduced to about 35 mmHg in both groups. Interruption of blood flow to the brain was documented by a

15-min period of local CBF values near zero (LD units) in all animals, indicating profound cerebral ischemia. This was followed by a short period of high lCBF during the first minutes of reperfusion (hyperperfusion). At 90 min of recovery, lCBF had returned to baseline levels in most animals. However, few animals continued to exhibit postischemic hypoperfusion at this time. MABP and lCBF were not different between groups at any time during insult and recovery.

Fig. 5 depicts superficial and basal brain temperature, as well as rectal temperature and lCBF, before and 2 min after the onset of the near-infrared radiation in animals of Group II. Shortly after extracranial heat application was initiated, superficial brain temperature was already significantly higher, but rectal and basal brain temperature remained unchanged (Fig. 5, panel A). In contrast, parallel lCBF measurements were not different when using the heating device (Fig. 5, panel B). EEG recordings were not influenced by heat application at any time (data not shown).

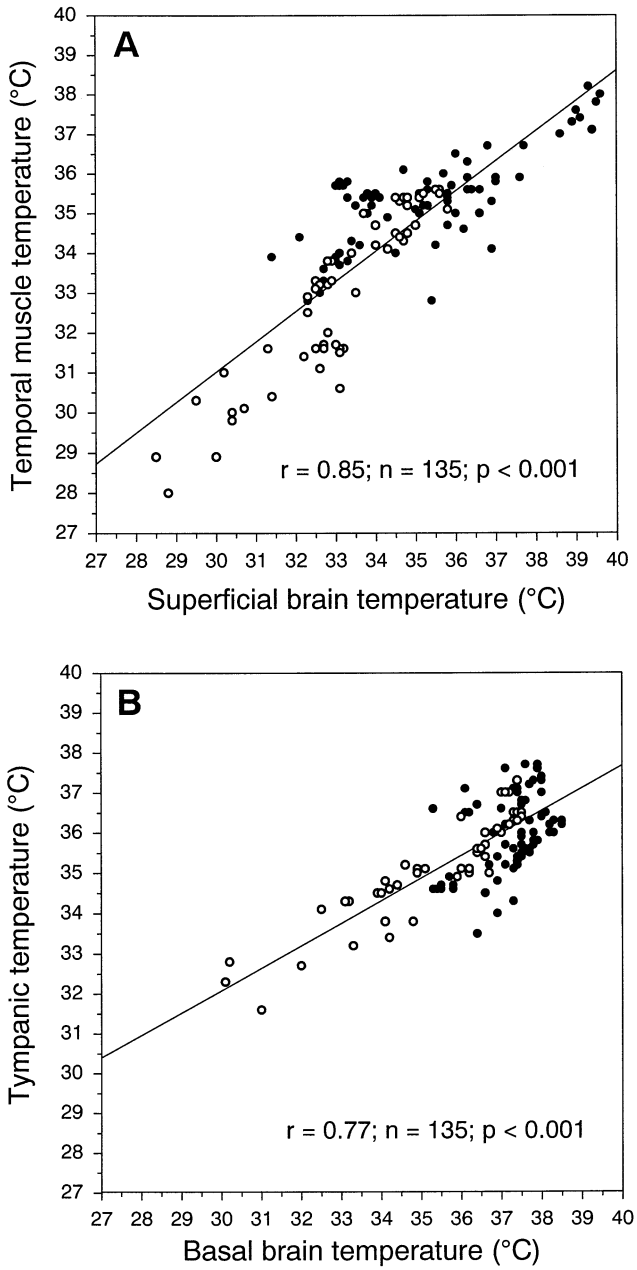

Fig. 4.

4. Discussion

The most important finding of the present study is that the novel near-infrared heat radiator is capable of maintaining peri-ischemic brain temperature within a narrow range in both superficial and basal areas, and does not interfere with optical or electrophysiological measurements, e.g. LDF or EEG. In addition, the new tympanal thermocouple probe closely reflects deep brain temperature and represents a non-invasive concept for brain temperature monitoring within small stereotaxic frames.

Our data suggest that exposure of the calvarium for various measurements renders the brain more susceptible to mild (at baseline) or moderate (during ischemia and early reperfusion) hypothermia if no additional heat source is used above the head. However, since peri-ischemic hypothermia is currently considered to be one of the few means available to reduce cellular damage, brain temperature needs to be meticulously controlled under conditions of experimental ischemia and reperfusion to prevent unwanted hypothermia.

Fig. 4. Plotted data represent single point temperature measurements ($n = 135$) for all animals included in the study ($n = 9$). Panel A shows the correlation between simultaneous temperature measurements of superficial brain areas (i.e. rostral hippocampus) and the temporal muscle [temporal muscle temperature = 8.197 + (0.760 * rostral hippocampal temperature)]; $r = 0.846$, $n = 135$; $P < 0.001$. Panel B presents the correlation between simultaneous temperature measurements in basal brain areas (i.e. occipital hippocampus) and the tympanic membrane [tympanal membrane temperature = 15.305 + (0.559 * occipital hippocampal temperature)]; $r = 0.768$, $n = 135$, $P < 0.001$. The calculated correlation coefficients for both sets of temperature measurements were higher for animals without extracranial heat application (open circles, group I) compared to those, in which the near-infrared heat radiator was used (closed circles, group II). Without EHA (group I, $n = 4$): [temporal muscle temperature without EHA = 1.584 + (1.043 * rostral hippocampal temperature without EHA)]; $r = 0.913$, $n = 60$, $P < 0.001$; [tympanal membrane temperature without EHA = 12.720 + (0.636 * occipital hippocampal temperature without EHA)]; $r = 0.912$, $n = 60$, $P < 0.001$; With EHA (group II, $n = 5$): [temporal muscle temperature with EHA = 19.570 + (0.447 * rostral hippocampal temperature with EHA)]; $r = 0.769$, $n = 75$, $P < 0.001$; [tympanal membrane temperature with EHA = 15.436 + (0.552 * occipital hippocampal temperature with EHA)]; $r = 0.466$, $n = 75$, $P < 0.001$.

4.1. Monitoring of brain temperature

In none of our experiments did the rectal temperature accurately reflect brain temperature when the exposed calvarium was without additional heat radiation. While core temperature was maintained thermostatically using a heating blanket at physiologic temperatures, the temperature of the exposed brain spontaneously decreased in these animals, resulting in major differences between rectal and brain temperatures. In contrast, differences were only minor when the new radiator was used. This is a result of the fact that in this study, the emitted energy of the device was regulated to maintain basal brain temperature at the same level as the core temperature (about 37.5°C). Furthermore, rectal temperature remained unchanged, when extracranial heat was applied or discontinued, while brain temperature in superficial and basal areas increased or decreased accordingly (Table 2, Figs. 2 and 3). This serves as additional evidence for the independence of brain and rectal temperatures in our setting, even with the use of the new heating device.

Our results confirm those obtained by others, showing that, depending on the specific situation, rectal temperature is an unreliable parameter of brain temperature (Busto et al., 1987; Minamisawa et al., 1990c; Jiang et al., 1991; Miyazawa and Hossmann, 1992). Without intervention, brain temperature was found to be about 1°C higher (van Rhoon and van der Zee, 1983; Mellergård and Nordström, 1990; Schwab et al., 1997; Henker et al., 1998), while it was significantly reduced with anesthesia (Jiang et al., 1991), and about 4–5°C lower during ischemia than rectal temperature (Busto et al., 1987; Minamisawa et al., 1990c; Miyazawa and Hossmann, 1992).

If brain temperatures are to be monitored, spontaneous temperature gradients within the brain have to be considered for the selection of the appropriate probe site, considering that deep brain structures, e.g. ventricles and striatal nucleus are warmer than superficial parts, e.g. the cortex, or the epidural space (Minamisawa et al., 1990c; Mellergård and Nordström, 1990; Mellergård, 1994, 1995; Schwab et al., 1997). Similarly, we observed low temperatures in superficial and higher temperatures in basal brain areas. In our experimental paradigm, basal brain temperature is closely reflected by the tympanic membrane temperature, and the superficial brain temperature by the temporal muscle temperature, respectively (Fig. 4). In contrast, there was no correlation between basal brain temperature (i.e. occipital hippocampus) and temporal muscle temperature in our study.

This parallels observations in humans, where the relationship between tympanal and epidural temperature appears to be very close (Mellergård and Nordström, 1990). The tympanic membrane was shown to reflect the temperature in the mesencephalon, even when temperature changes occurred during brain exposure (Mariak et al., 1994). Our data suggest that the non-invasive measurement of tympanic membrane temperature is suitable for basal brain temperature monitoring in rats. It can replace more invasive techniques, e.g. epidural temperature probes, especially when the calvarium is to remain intact for long-term postischemia outcome studies. However, it should be taken into consideration that tympanic membrane temperature measurements may underestimate the actual temperature of basal brain structures by about 1°C (Fig. 2, panel B; Mariak et al., 1993) and may be misleading during periods of rapid temperature changes.

Table 2
Rectal temperature, mean arterial blood pressure and local cerebral blood flow before, during and after ischemia[a]

		Baseline		Ischemia		Reperfusion			
		−7 min	−1 min	2 min	15 min	3 min	10 min	30 min	60 min
Rectal temperature (°C)	Without EHA	37.5 ± 0.03	37.4 ± 0.04	37.3 ± 0.05	36.6 ± 0.3	36.8 ± 0.2	36.9 ± 0.3	37.7 ± 0.03	37.5 ± 0.1
	With EHA	37.4 ± 0.08	37.4 ± 0.1	36.9 ± 0.2	35.1 ± 0.5*	36.5 ± 0.2	37.3 ± 0.2	37.9 ± 0.3	37.6 ± 0.2
MABP (mmHg)	Without EHA	78.6 ± 3.7	77.3 ± 3.7	33.6 ± 0.6	34.6 ± 0.3	68.3 ± 2.7	76.9 ± 4.5	76.9 ± 3.5	79.7 ± 3.2
	With EHA	77.5 ± 4	75 ± 6.6	33.6 ± 0.8	35.8 ± 1.1	74.9 ± 6.2	80.9 ± 6.8	76.5 ± 4.8	82.1 ± 7.9
Local CBF (units)	Without EHA	32.4 ± 3.7	31.1 ± 3.3	4.4 ± 1.1	2.6 ± 0.5	21.2 ± 7.7	39.9 ± 7.7	30.1 ± 3.1	26.2 ± 3.2
	With EHA	36.3 ± 6.1	35.9 ± 6.3	4.3 ± 0.8	2.2 ± 0.7	21.3 ± 7.2	50.6 ± 14.6	39.7 ± 10.4	37.8 ± 8.6

[a] Data are mean ± SEM. No statistically significant differences between groups, except rectal temperature at 15 min ischemia, which was lower with EHA than without; (* Student's t-test, $P = 0.045$). EHA, extracranial heat application; MABP, mean arterial blood pressure; CBF, cerebral blood flow.

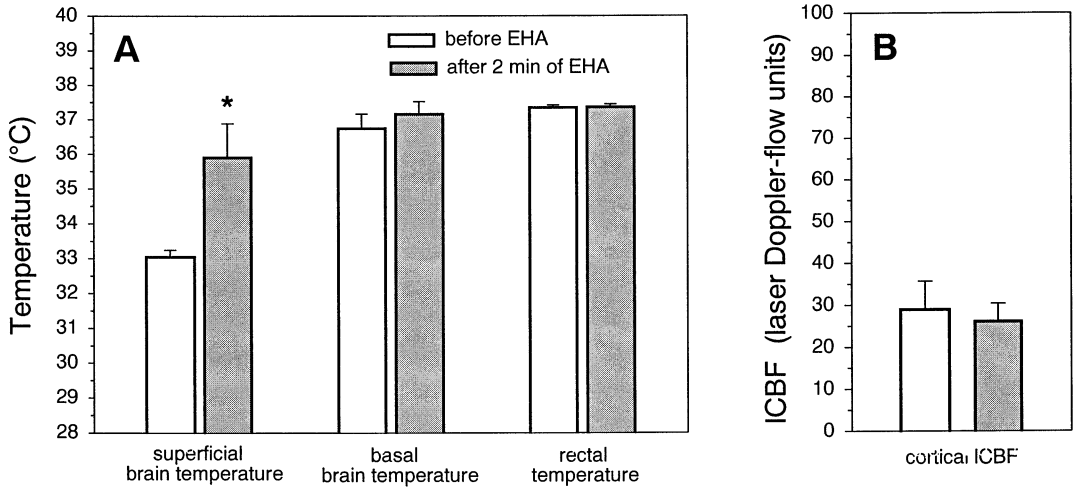

Fig. 5. Superficial, basal brain and rectal temperature (panel A), as well as local CBF (panel B) before and 2 min after the onset of near-infrared radiation for animals of Group II ($n = 5$). Data are mean ± SEM. Two minutes after the onset of additional heat radiation superficial brain temperature was already significantly higher than before ($P < 0.05$, paired t-test). The pre-ischemic temperature of 33.0 ± 0.2 was significantly below the physiologic range following craniotomy. This clearly illustrates the need for additional extracranial heat application. Basal brain and rectal temperatures were unchanged. Local cerebral blood flow was not influenced by the onset of the near-infrared heat radiation (panel B).

The specific advantage of the new thermocouple probe may be seen in the fact that tympanal membrane temperature can be obtained within a stereotaxic apparatus.

4.2. Maintenance of brain temperature

The near-infrared radiator maintained brain temperature in both superficial and basal areas of the brain within the desired (physiologic) range, as confirmed by simultaneous direct brain temperature measurements in the rostral and occipital hippocampus. Spontaneous temperature gradients observed in animals without extracranial heat application were avoided.

Placing the animals on a thermostatically-controlled heating blanket alone did not prevent the occurrence of brain hypothermia in our experimental setting. A conventional heating lamp directed towards the skull or superfusion of the calvarium with warm saline, al though known to prevent isolated brain temperature loss during ischemia and reperfusion (Busto et al., 1987; Minamisawa et al., 1990c; Colbourne et al., 1993), could not be applied in our experimental setup, because continuous optical and electrophysiologic measurements were to be obtained in parallel.

As previously shown for microwave irradiation (Ward et al., 1986), electromagnetic wavelengths invisible to the human eye may be used to influence brain temperature. Near-infrared radiation may interfere with simultaneous optical measurements using wavelengths close to those applied for warming the brain, e.g. LDF probes ($\lambda = 780$ nm) for continuous CBF-measurements. The emitted spectrum of the new near-infrared radiofrequency generator ($\lambda = 800$ nm to > 10 μm) theoretically does not overlap with the LDF wavelength. Even in the presence of slight overlapping, at an energy peak of $\lambda = 3.5$ μm only very small portions of the transmitted energy might interfere with the LDF measurement. In our setting, LDF-signals were not different when recorded with and without the new device in the same animals (Fig. 5B).

In addition to careful observation and meticulous maintenance of intraischemic brain temperature, attention needs to be paid to temperature control after brain insults when animals are observed for prolonged periods to assess long-term outcome.

Postischemic hypothermia appears to reduce brain injury (Busto et al., 1989; Buchan and Pulsinelli, 1990; Chopp et al., 1991; Colbourne and Corbett, 1994, 1995; Coimbra et al., 1996; Corbett et al., 1997; Johnston, 1997; Laptook et al., 1997; Nakajima et al., 1997; Thoeresen et al., 1997; Trescher et al., 1997), although no long-term benefit has been observed for short post-insult periods of hypothermia (e.g. < 3 h, Welsh and Harris, 1991; Dietrich et al., 1993). It has been suggested that some assumedly neuroprotective interventions, e.g. glutamate receptor antagonists, GABA re-uptake inhibition, volatile anesthetics, adrenalectomy, act primarily through their ability to reduce body temperature during postischemic recovery (Kuroiwa et al., 1990; Morse and Davis, 1990; Inglefield et al., 1995; Ide et al., 1996; Nurse and Corbett, 1996; Britton et al., 1997; Gilland and Hagberg, 1997; Zhang et al., 1997). Complex methods as proposed for control of cerebral temperature in freely moving animals (Colbourne et al., 1996; Zhang et al., 1997) are suggested for use during the post-insult recovery period in pharmacological long-term studies.

In conclusion, our data show that the described non-invasive approach comprising multiple extracranial measurement sites and additional heat radiation improves brain temperature control during ischemia, enabling maintenance of designated temperatures in all parts of the brain. The method appears to be especially useful for experimental procedures, which include simultaneous and continuous optical and electrical measurement techniques for parallel monitoring of multiple organ functions. Furthermore, the non-invasive approach is recommended for the perioperative period in long-term studies of postischemic outcome.

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