Cerebral tumor necrosis factor α expression and long-term neurocognitive performance after cardiopulmonary bypass in rats

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Objective: Cerebral inflammatory reaction is discussed as a contributor to adverse cerebral outcome after cardiac surgery with cardiopulmonary bypass. This study was designed to determine the effect of cardiopulmonary bypass on both cerebral expression of tumor necrosis factor α and neurocognitive outcome in rats.

Methods: With institutional review board approval, 50 rats were randomly assigned to one of 3 groups: rats of the cardiopulmonary bypass group were subjected to 75 minutes of normothermic cardiopulmonary bypass. Sham-operated animals underwent identical preparation but were not connected to cardiopulmonary bypass, whereas rats of the control group were neither anesthetized nor cannulated. Ten rats per group survived 4 hours after cardiopulmonary bypass or the sham operation for immediate postoperative determination of tumor necrosis factor α -expressing cells (immunohistochemistry) and cerebral tumor necrosis factor α mRNA levels (polymerase chain reaction). The remaining animals survived 10 days for neurocognitive assessment by using the modified hole-board test and for analysis of cerebral tumor necrosis factor α activation in the late postoperative period.

Results: Expression of tumor necrosis factor α mRNA was increased 4 hours after cardiopulmonary bypass and the sham operation, with higher expression in the cardiopulmonary bypass group ($\chi^2 [2] = 25.08, P < .001$). Both experimental groups demonstrated larger numbers of tumor necrosis factor α -positive cells in the early and late postoperative periods ($F [1] = 13.08, P \leq .001$) and an impaired neurocognitive performance on the first postoperative days compared with that seen in the control group ($F [2, 24] = 4.26, P = .02$).

Conclusions: Cerebral tumor necrosis factor α activation in both experimental groups during the early postoperative period was accompanied by transient neurocognitive impairment. Therefore cardiopulmonary bypass alone demonstrated no effect on cerebral inflammation and neurocognitive outcome.

Although considerable advancements in surgical and anesthetic techniques have improved overall morbidity and mortality in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB), neurologic and neurocognitive dysfunction remain as common and long-lasting complications.¹ The cause of these adverse outcomes is likely multifactorial, including mainly the effects of microembolization and global cerebral hypoperfusion next to systemic inflammation.² The inflammatory cascade is specifically activated during CPB through several pathways: the contact activation by the foreign surface of the circuit, surgical trauma, and the effect of ischemia-reperfusion injury and endotoxemia.^{3,4} Human studies examining the effect of systemic inflammatory reaction on cerebral outcome after CPB have revealed conflicting results.^{3,5,6} The advantage of an animal model

is to analyze the inflammatory response directly in the brain and consequently its effect on neurocognitive outcome. An important step forward was the study by Hindman and colleagues⁷ demonstrating a link between systemic interleukin 6 and cerebral cyclooxygenase 2 mRNA expression, but the time course of cerebral inflammation and the association with neurocognitive outcome after CPB have not been studied thus far.

Therefore this study was performed to investigate the effect of CPB on (1) cerebral expression of tumor necrosis factor (TNF) α and (2) neurocognitive outcome. Furthermore, as secondary end points, we investigated levels of cerebral TNF- α mRNA and behavioral outcome because these parameters might influence the primary end points.

MATERIALS AND METHODS

Study Design

All animals were treated in compliance with the "Principles of laboratory animal care" formulated for the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the National Academy of Sciences (National Institutes of Health publication no. 86-23, revised 1985). Experimental protocols were approved by the institutional animal care committee (Regierung von Oberbayern, Munich, Germany).

Rats were randomly assigned to one of 3 groups: rats in the CPB group were subjected to 75 minutes of normothermic CPB ($n = 20$), whereas rats in the sham-operated group were exposed to the same surgical preparation

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Abbreviations and Acronyms

CPB = cardiopulmonary bypass

MAP = mean arterial pressure

and anesthesia but not connected to CPB ($n = 20$). Rats in the naive control group were transferred to the laboratory to mimic the stress of transport and isolation from the familiar group but were neither anesthetized nor surgically prepared ($n = 10$). Ten rats of the CPB and sham-operated groups were assigned to short-term survival (4 hours of recovery), allowing for the determination of cerebral TNF- α mRNA levels and the amount of TNF- α -expressing hippocampal neurons and astrocytes. All other animals were allowed to survive long-term to assess postoperative neurocognitive performance and behavior by using the modified hole-board test next to the determination of cerebral TNF- α levels at postoperative day 10 (Figure 1).

Surgical Preparation and CPB

Nonfasted male Sprague–Dawley rats from Charles River Laboratories (Sulzfeld, Germany; 375 ± 36 g, 10 weeks old) were anesthetized with 3% isoflurane in air and intubated. Ventilation was adjusted to maintain a P_{aCO_2} of 35 to 45 mm Hg, and anesthesia was maintained with 2.0% to 2.5% isoflurane and repetitive boluses of fentanyl ($5 \mu\text{g}$). Subsequently, all rats of the CPB and sham-operated groups were cannulated as previously reported by using aseptic techniques.⁸ Briefly, the tail artery was cannulated for aortic inflow, the right external jugular vein for venous return, and the right superficial caudal epigastric artery for monitoring of mean arterial pressure (MAP). After cannulation of the tail artery, 150 IU heparin was administered. Pericranial temperature was monitored and controlled to 37.5°C (HYP-1; Newport, Santa Ana, Calif) by using a heating blanket and convective forced-air heating system. Serum glucose concentration was controlled at greater than 80 mg/dL, administering respective amounts of glucose 50% as required. Arterial blood gas measurements were performed with a blood gas analyzer (Rapidlab 860; Bayer Vital GmbH, Fernwald, Germany).

The CPB circuit consisted of a venous reservoir, peristaltic pump (Masterflex; Cole-Parmer Instrument Co, Vernon Hills, Ill), custom-designed membrane oxygenator, inline flow probe (2N806 probe and T208 flowmeter; Transonics Systems, Inc, Ithaca, NY), and arterial inflow cannula. The rat oxygenator is built of 2 Plexiglas shells ($12.8 \text{ cm} \times 12.8 \text{ cm} \times 2.7 \text{ cm}$) carrying the diffusion membrane and consisting 3 layers of polypropylene hollow-fiber mats (Jostra AG, Hirrlingen, Germany) glued together in a crosswise fashion to improve oxygenation. The provided gas exchange area is 558 cm^2 , and the prime volume of the oxygenator is 4 mL. An integrated water bath allows heating the circuit and helps avoid a decrease in the body temperature of the rat throughout extracorporeal circulation. The complete CPB circuit (10 mL) was primed with 8 mL of whole blood obtained from 1 heparinized (150 IU/rat) donor rat, 2 mL of 6% hetastarch, and 100 IU of heparin.

After preparation, rats of the CPB groups were subjected to 75 minutes of normothermic, nonpulsatile CPB with flow rates of 160 to $180 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. During CPB, ventilation of the lungs was terminated. Anesthesia was maintained with 0.8% to 1.2% isoflurane administered through the oxygenator, subsequent doses of $5 \mu\text{g}$ of fentanyl as required, and 1.6 mg/h cisatracurium. Sham-operated animals were exposed to the same anesthetic conditions for 75 minutes but not connected to the CPB circuit. After 75 minutes, animals in the CPB group were weaned from CPB without any inotropic support, and all animals were decannulated. The heparin anticoagulation was allowed to dissipate spontaneously without supplemental administration of protamine.

After decannulation, rats assigned to short-term recovery remained anesthetized, intubated, and ventilated for 4 hours after cessation of CPB. After obtaining the final set of hemodynamic measurements, the isoflurane con-

centration was increased to 5%, and the brains were removed and frozen in tissue-freezing medium (Leica Instruments GmbH, Nussloch, Germany) with methylbutane and stored at -80°C for further analyses. Rats assigned to survive for 10 days (long-term survival) remained anesthetized with 1.5% to 2% isoflurane (without additional fentanyl or cisatracurium) and were intubated and ventilated for 2 hours. When animals resumed spontaneous ventilation, the tracheas were extubated. The animals were allowed to recover in an oxygen-enriched environment for 2 to 4 hours with free access to water and food. Thereafter, they were returned to their home-board cages and housed in familiar groups. Rats in the untreated control group stayed in the laboratory with free access to water and food for the identical period to mimic hospitalization.

Neurocognitive Testing

Cognitive and behavioral parameters were assessed with the modified hole-board test by a dedicated investigator blinded to treatment. Rats were housed under standard laboratory conditions (12 hours of light/12 hours of dark, lights on at 12:30 AM, 22°C , and 60% humidity, with free access to water and standard rat chow) 3 weeks before the experiments to acclimate to the changed day/night rhythm. Nine days before neurocognitive testing, animals were housed in the modified hole-board environment for habituation. Subsequently, rats learned the test procedure during 10 consecutive days (training phase). The next day, the animals underwent surgical preparation with or without CPB or served as control animals, followed by a postoperative test period of 10 days.

The modified hole-board test was performed according to an established protocol.^{9,10} Briefly, the rats lived in an area divided into a home cage ($80 \times 60 \times 50 \text{ cm}$) and a test arena ($40 \times 60 \times 50 \text{ cm}$), with the hole board ($20 \times 40 \text{ cm}$) placed in the middle of the arena. Fifteen holes covered by lids were staggered on the board. After opening, coil springs force the lids back to their original position. Three holes baited with puffed rice were marked with white tape. All holes were flavored with black currant aroma to cover the odor of the puffed rice. Animals were tested with the modified hole-board test for 3 trials daily. The sequence of marked holes was randomly changed daily.

Three cognitive parameters were evaluated: (1) deficit in overall cognitive performance was assumed if the time needed to complete 1 trial was extended compared with that among untreated control animals; (2) deficit within the visuospatial long-term memory was assumed if rats visited non-baited holes or did not visit baited holes, which was referred to as wrong choice; and (3) deficit within the visuospatial short-term memory was assumed if rats revisited a baited hole, which was referred to as repeated choice. Additionally, aspects of behavior, such as motivation, anxiety, and exploration, were assessed.¹⁰

After completion of the neurocognitive testing on postoperative day 10, animals were anesthetized with 5% isoflurane, and the brains were removed and frozen in tissue-freezing medium (Leica Instruments GmbH) and stored at -80°C for further analyses.

Immunohistochemistry and Reverse Transcription–Polymerase Chain Reaction

Frozen brains were cut into $10\text{-}\mu\text{m}$ slices at bregma -3.3 mm by using a cryotome (Microtom-Kryostat, HM 500 QM; Microtome, Walldorf, Germany). Adjacent slices were subjected to standard histology and immunohistochemistry, and the remaining tissue was used to perform reverse transcription–polymerase chain reaction.

By using immunohistochemical techniques, frozen sections were double stained for TNF- α (polyclonal rabbit anti-rat TNF- α ; Acris Antibodies GmbH, Herford, Germany) and NeuN (Mouse Anti-Neuronal Nuclei Monoclonal Antibody; Chemicon International, Temecula, Calif) or glial fibrillary acidic protein (Mouse Monoclonal Anti-Glial Fibrillary Acidic Protein; Chemicon International), respectively. TNF- α was labeled dark brown by using a biotinylated secondary antibody, peroxidase, and diaminobenzidine. Neurons or astrocytes were marked bright red by using an alkaline

FIGURE 1. Diagram of the experimental design. Three groups were studied: the cardiopulmonary bypass (CPB) group, the sham-operated group, and the control group. Short-term groups survived 4 hours, whereas long-term groups survived 10 days. End points were as follows: polymerase chain reaction (PCR), immunohistochemistry (IHC), hematoxylin and eosin staining (HE), and, in the long-term groups, results of the modified hole-board test (mHBT).

phosphatase-conjugated secondary antibody and Vector Red alkaline phosphatase substrate kit (Vector Laboratories, Burlingame, Calif). The number of TNF- α -positive cells (neurons and astrocytes) was counted in the hippocampus by using light microscopy (440-fold magnification).

Total RNA was extracted with the Rneasy Mini Kit (Qiagen, Inc, Hilden, Germany), followed by reverse transcription of RNA (4 μ g) to cDNA with random hexamers. By using rat primers for TNF- α and β -actin, cDNA was coamplified (28 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1.5 minutes for TNF- α and 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute for β -actin). Specific rat primers were as follows: TNF- α , sense primer CTCAAACTCGAGTGACAAGC and antisense primer CCG TGATGTCTAAGTACTTGG [NM_012675]; β -actin, sense primer GTGGGGCGCCCCAGGCACCA and antisense primer CTCCTTAATGTCACGCACGATTTC [BT019932]. The polymerase chain reaction products were subjected to electrophoresis in 1.8% agarose, stained with ethidium bromide, and photographed. The predicted lengths of amplification products for TNF- α and β -actin were 419 and 548 bp. Results are presented as ratios of band intensities of TNF- α mRNA to β -actin mRNA (Quantity One Quantification software 4.1; Bio-Rad Laboratories, Hercules, Calif).

For standard histology, slices were stained with hematoxylin and eosin. Five high-magnification fields (440-fold) in each of 4 brain regions (striatum, motorcortex, cingulate cortex, and hippocampus) were viewed by an investigator blinded to group assignment to assess neuronal damage.

Statistical Analysis

Physiologic parameters (MAP, hemoglobin concentration, P_{aCO_2} , and P_{aO_2}) and cognitive and behavioral values were analyzed by using general linear models with the between-group factor preparation (CPB vs sham vs control), the within-group factor time, and their interaction term (time $\times$ preparation). Effects of time levels were analyzed quadratically (time²) for physiologic, cognitive, and behavioral values, focusing on biphasic changes of these variables during the respective observation time (operation minutes and preoperative or postoperative days, respectively). Values were analyzed post hoc by using factorial analyses of variance, followed by Bonferroni *t* tests at each time point. Cerebral TNF- α mRNA expression was analyzed by using nonparametric analysis of variance (Kruskal–Wallis–Test) with post hoc (given that $P < .05$) Mann–Whitney *U* tests ($P < .05$). Glial and neuronal TNF- α protein expression was analyzed by using a 1-way analysis of variance. Where analyses of variance were significant, post hoc comparisons were performed with Tamhane’s test of significance.

RESULTS

Four rats of the CPB group died of technical problems (cannulation problems or insufficient venous return) and were replaced to keep the sample size equal.

Rats subjected to CPB demonstrated lower MAP (F [1, 36] = 16.87, $P \leq .001$), lower hemoglobin concentrations (F [1, 35] = 5.62, $P = .023$), and higher P_{aO_2} values during CPB (F [1, 36] = 28.18, $P \leq .001$) than rats of the sham-operated groups, most likely because of the nature of CPB (Table 1).

Immunohistochemical double staining demonstrated a larger number of TNF- α -positive neurons (CPB/long, 17.3 ± 5.6 ; CPB/short, 11.7 ± 2.3 ; sham/long, 16.5 ± 7.4 ; sham/short, 12.2 ± 2.2) and astrocytes (CPB/long, 15.5 ± 3.6 ; CPB/short, 12.0 ± 3.2 ; sham/long, 17.1 ± 6.1 ; sham/short, 11.7 ± 3.6) 4 hours (short) or 10 days (long) after CPB and the sham operation compared with control animals (neurons, 2.8 ± 1.8 ; astrocytes, 0.9 ± 0.9), with no difference between the CPB and sham-operated groups ($P \leq .001$ and for TNF- α -positive astrocytes: F [4] = 25.76, $P \leq .001$; Figure 2).

Cerebral expression of TNF- α mRNA was increased 4 hours after CPB (ratio of TNF- α to β -actin mRNA, 0.089 ± 0.03) and the sham operation (ratio of TNF- α to β -actin mRNA, 0.080 ± 0.02), with higher levels in rats subjected to CPB (χ^2 [2] = 25.08, $P < .001$), whereas no cerebral

TABLE 1. Physiologic data during the operative procedure in the 2 experimental groups

	Preparation	Pre-CPB	45 min CPB	75 min CPB	2 h post-CPB
Mean arterial pressure (mm Hg)	Sham	79.4 $\pm$ 1.4	75.0 $\pm$ 1.4*	70.5 $\pm$ 1.7*	74.4 $\pm$ 1.7
	CPB	81.4 $\pm$ 1.3	66.0 $\pm$ 2.8	63.4 $\pm$ 2.2	73.6 $\pm$ 1.4
Hemoglobin concentration (mg/dL)	Sham	14.0 $\pm$ 0.2	13.3 $\pm$ 0.3*	12.5 $\pm$ 0.2	11.7 $\pm$ 0.3*
	CPB	14.3 $\pm$ 0.2	12.1 $\pm$ 0.2	11.9 $\pm$ 0.2	10.8 $\pm$ 0.3
P_{aCO_2} (mm Hg)	Sham	38.3 $\pm$ 1.2	38.6 $\pm$ 0.9	38.6 $\pm$ 0.9	37.5 $\pm$ 0.7
	CPB	40.2 $\pm$ 0.8	42.2 $\pm$ 1.3	38.3 $\pm$ 0.6	35.7 $\pm$ 1.0
P_{aO_2} (mm Hg)	Sham	182 $\pm$ 9	191 $\pm$ 7*	195 $\pm$ 12*	197 $\pm$ 8
	CPB	165 $\pm$ 6	274 $\pm$ 18	289 $\pm$ 17	178 $\pm$ 12

Variables were obtained before cardiopulmonary bypass (Pre-CPB), at 45 minutes of cardiopulmonary bypass (45 min CPB), at 75 minutes of cardiopulmonary bypass (75 min CPB), and 2 hours after cardiopulmonary bypass (2 h post-CPB) or to equivalent times in the sham-operated groups (Sham). Values are presented as means $\pm$ standard errors of the mean. * $P < .05$ versus cardiopulmonary bypass.

FIGURE 2. Immunohistochemistry of coronal brain sections 4 hours and 10 days after cardiopulmonary bypass (CPB) and the sham operation (Sham). The number of tumor necrosis factor α (TNF- α)-positive neurons (A) and astrocytes (B) in the hippocampus is increased in all 4 groups compared with that seen in untreated control animals (* $P < .05$ vs untreated control group). The dashed line marks the upper 95% confidence interval of the untreated control animals.

TNF- α mRNA was detected in untreated control animals (ratio of TNF- α to β -actin mRNA, 0.0 ± 0.0) and in all animals on postoperative day 10 (Figure 3).

Histologic assessment revealed no significant neuronal cell damage in any of the groups 4 hours after CPB or the sham operation or 10 days postoperatively.

Overall cognitive performance was impaired during the first 4 postoperative days after CPB and the sham operation compared with that seen in native control rats without any difference between the CPB and sham-operated groups. This transient dysfunction was not accompanied by impaired long-term or short-term memory (for the parameter time trial: $F [2, 24] = 4.26$, $P = .02$; Figure 4). No differences in behavior were detected during the observation period.

DISCUSSION

The current study demonstrates that CPB does not aggravate cerebral TNF- α expression and does not cause longer-lasting neurocognitive impairment when compared with that seen in sham-operated rats.

In the past, several animal models of cerebral ischemia have provided important insights into the mechanisms of in-

FIGURE 3. Tumor necrosis factor α (TNF- α) mRNA expression in the brain 4 hours after cardiopulmonary bypass (CPB) and the sham operation compared with that seen in control animals. TNF- α mRNA/ β -actin mRNA ratios were increased after both CPB and the sham operation, with higher expression in the CPB group. * $P < .05$ vs untreated control animals; § $P < .05$ versus the sham group.

flammatory response in the brain after cerebral injury with respect to inflammatory gene expression, inflammatory protein synthesis, and regional distribution of neurons expressing cytokines, particularly the proinflammatory cytokine TNF- α .^{11,12} Experimentally, TNF- α has been shown to enhance the permeability of the blood-brain barrier; to induce expression of endothelial adhesion molecules, leading to further release of inflammatory mediators; and to be involved in thrombogenic and vascular changes after brain injury.^{13,14} Clinically, TNF- α levels have been shown to increase in response to cardiac surgery with CPB.⁴ Therefore we selected TNF- α as an inflammatory marker in the current study to investigate the effect of CPB on cerebral inflammation in rats. Surprisingly, cerebral expression of TNF- α protein in neurons and astrocytes of the hippocampus was comparable between the CPB and the sham-operated groups, supposing no effect of CPB alone without major surgical intervention in young and healthy rats undergoing cerebral inflammation. Not only was the expression of the protein investigated but also the gene expression as levels of mRNA to perform a comprehensive analysis of TNF- α as an inflammatory marker. These levels were higher in both experimental groups when compared with those in the control group, with a significant, although minor, increase in the CPB group (Figure 3). One can only speculate whether compensatory anti-inflammatory parameters

FIGURE 4. Neurocognitive function of rats at various times before and after cardiopulmonary bypass (CPB) and the sham operation. Overall cognitive performance (time needed to complete the test; A) was impaired on the first 4 postoperative days after CPB and the sham operation ($P < .05$) but improved over time and reached baseline values on postoperative day 6 ($P < .05$). Visuospatial long-term memory (number of wrong choices; B) was comparable among groups ($P > .05$). Visuospatial short-term memory (number of repeated choices; C) was comparable among groups ($P > .05$). OP, Operation.

might have prevented the successful translation of TNF- α mRNA to protein in rats of the CPB group.¹⁵ However, TNF- α protein rather than TNF- α mRNA has been chosen as a major end point in this study because the protein and not the gene influences the subsequent inflammatory cascade.

Of note, numbers of TNF- α -expressing neurons and astrocytes are still increased in the hippocampus 10 days after CPB and the sham operation when compared with those seen in untreated control animals. Previous experimental studies have demonstrated TNF- α expression in the brain peaking between 12 and 36 hours after cerebral ischemia, with an attenuated response on postoperative day 10,^{16,17} whereas the microglial response peaked between 4 and 7 days after cerebral injury.¹⁸ Relating to the results of the current study, we speculate that the early postoperative TNF- α release at 4 hours reflects the onset of the inflammatory response only. TNF- α -expressing cells detected in the late postoperative period could then represent the remnants of this inflammatory reaction, which is substantiated by the absence of TNF- α mRNA 10 days after CPB and the sham operation. Both positive and negative controls were included in the reverse transcription-polymerase chain reaction protocols to rule out methodological problems.

Whether the pronounced inflammatory reaction is caused by anesthesia or the surgical preparation was not investigated in the current study. On the one hand, human studies revealed conflicting results regarding the effect of surgical approach on systemic inflammatory reaction.^{19,20} On the other hand, studies about the influence of anesthesia on inflammation suggest a reduction in cytokine release.^{21,22}

Rats of both the CPB and the sham-operated groups had a transient decrease in cognitive performance, however, without any specific cognitive dysfunction, such as impaired long-term or short-term memory. This unspecific cognitive deficit lasted just until postoperative day 6. In addition, behavioral dimensions did not show any distinctive feature with respect to anxiety or motivation. Taking these results together, anesthesia, surgical preparation, or both rather than CPB might have caused a cognitive slowing during the first postoperative days.

These results are in agreement with those of other studies showing similar short- and long-term cognitive outcomes in CPB versus sham-operated rats,^{23,24} as well as with some clinical studies demonstrating that coronary artery bypass grafting with and without CPB resulted in similar incidences of neurocognitive dysfunction.²⁵ However, other experimental studies with this CPB model have demonstrated short-term cognitive deficits after CPB when compared with sham-operated rats.^{8,26} One difference between those studies and the current study is the use of the modified hole-board test to assess neurocognitive performance. This test presents a unique test battery that allows a comprehensive and detailed

evaluation of cerebral outcome in the rat. This test offers a sensitive testing environment, allowing for the assessment of a variety of cognitive and behavioral parameters in only 1 test.⁹ Specifically, this test battery allowed a complete evaluation of neuropsychologic performance until postoperative day 10 compared with a preoperatively established baseline. In addition, behavioral aspects, such as anxiety and motivation, were determined because it is well known that these parameters can influence cognitive function.¹⁰

However, important limitations remain. Median sternotomy, thoracotomy, direct cardiac cannulation, and cardiac surgery were not performed to allow the long-term survival of the animals. Therefore the marked inflammatory response generated by the surgical trauma or the reinfusion of blood from cardiectomy suction into the CPB circuit and its effect on cerebral inflammation and cognitive outcome could not be studied with this particular model. Other limitations include the fact that CPB was conducted under conditions of normothermia to exclude any effects of temperature on cerebral outcome. Also, young healthy rats, as used for this study, naturally lack any of the comorbidities and significant atherosclerotic disease found in the aging patient population presenting for cardiac surgery. Lastly, these adolescent animals might be able to better compensate for at least minor postoperative cognitive dysfunction and recover quicker and easier compared with typical cardiac patients. Based on the current model and results, we cannot identify the final cause of inflammatory activation, cognitive slowing, or both. Because we did not include a group receiving anesthesia without undergoing an operation, we cannot discriminate the effect of preparation or anesthesia on inflammatory response, cognitive outcome, or both. However, the aim of this study was to investigate the additional influence of CPB on cerebral inflammation and not the origin of inflammatory activation.

In summary, in this animal study increased cerebral TNF- α expression during the early postoperative period after CPB and the sham operation was accompanied by transient neurocognitive impairment without any difference between the groups. These results suggest that CPB alone in the absence of any other potential risk factors (embolism, aortic manipulation, or comorbidities) does not lead to a pronounced cerebral TNF- α expression or longer-lasting neurocognitive deficits after cardiac surgery.

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