Anesthesiology 2001; 95:1485–91 

# *Cardiopulmonary Bypass Induces Neurologic and Neurocognitive Dysfunction in the Rat*

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***Background:*** **Neurocognitive dysfunction is a common complication of cardiac surgery using cardiopulmonary bypass (CPB). Elucidating injury mechanisms and developing neuroprotective strategies have been hampered by the lack of a suitable long-term recovery model of CPB. The purpose of this study was to investigate neurologic and neurocognitive outcome after CPB in a recovery model of CPB in the rat.**

***Methods:*** **Fasted rats (n = 10) were subjected to 60 min of normothermic (37.5°C) nonpulsatile CPB using a roller pump and a membrane oxygenator. Sham-operated controls (n = 10) were not subjected to CPB. Neurologic outcome was assessed on days 1, 3, and 12 after CPB using standardized functional testing. Neurocognitive outcome, defined as the time (or latency) to finding a submerged platform in a Morris water maze (an indicator of visual–spatial learning and memory), was evaluated daily from post-CPB days 3–12. Histologic injury in the hippocampus was also evaluated.**

***Results:*** **Neurologic outcome was worse in the CPB *versus* the sham-operated controls at all three measurement intervals ($P < 0.001$). The CPB group also had longer water maze latencies compared with the sham-operated controls ($P = 0.004$), indicating significant neurocognitive dysfunction after CPB. No difference in histologic injury between groups was observed.**

***Conclusions:*** **CPB caused both neurologic and neurocognitive impairment in a rodent recovery model. This model could potentially facilitate the investigation of CPB-related injury mechanisms and possible neuroprotective interventions.**

SINCE the advent of cardiopulmonary bypass (CPB), cerebral injury after cardiac surgery has been repeatedly documented in humans.[1–3] Clinical manifestations of this injury are variable, ranging from frank stroke to subtle neurocognitive dysfunction.[4,5] Although these types of injuries have been studied in humans for decades, the mechanisms of injury are incompletely described.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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Received from the Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina. Submitted for publication March 8, 2001. Accepted for publication August 7, 2001. Supported by a postdoctoral stipend from the German Academic Exchange Service, Bonn, Germany; a Society of Cardiovascular Anesthesiologists/Ischemia Research and Education Foundation Starter Grant, Richmond, Virginia (both to Dr. Mackensen); National Research Service Award No. GM08600-03 from the National Institutes of Health, Bethesda, Maryland; and Duke University Claude D. Pepper older Americans Independence Center Junior Faculty Award No. NIA-AG-11268 (both to Dr. Grocott).

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A major limitation to investigating injury mechanisms and potentially developing neuroprotective strategies has been the lack of a reproducible animal model of cerebral injury after CPB.[6] Although numerous animal models of CPB have been described,[7–9] they have substantial limitations. Models of CPB using large animals require sophisticated surgical expertise and have examined only short recovery intervals. Such models are also expensive, particularly with respect to management of extended recovery from CPB. Large animals are also limited by lack of development of standardized neurologic and neurocognitive measurement techniques.

The rat has been extensively used for the investigation of cerebral injury, allowing a wide spectrum of well-developed methods for definitions of mechanisms and neuroprotective interventions in a number of different settings, including global and focal cerebral ischemia.[10–15] Because of this, we sought to determine whether the rat could become a suitable recovery model for the study of cerebral dysfunction associated with CPB. The purpose of this investigation was to determine (1) whether long-term (> 24 h) survival in a rat CPB model is feasible, (2) whether exposure to CPB causes neurologic or neurocognitive dysfunction in rats, and (3) whether such dysfunction is associated with specific histologic findings.

## Methods

The following experimental protocol was approved by the Duke University Animal Care and Use Committee (Durham, NC). All procedures herein described met the guidelines of the National Institutes of Health for animal care.[16]

Male Sprague-Dawley rats (age, 12–14 weeks; weight, 325–375 g; Harlan, Indianapolis, IN) were fasted for 12–16 h but had free access to water. The animals were then anesthetized with 3% isoflurane in oxygen in a plastic box. After orotracheal intubation, the lungs were mechanically ventilated (30% $O_2$–balance $N_2$). Ventilation was adjusted to maintain an arterial carbon dioxide tension ($Paco_2$) of 36-42 mmHg. During subsequent surgical preparation, anesthesia was maintained with 2.0–2.5% isoflurane. Surgery was performed with an aseptic technique, and all surgical fields were subsequently infiltrated with 1% lidocaine. Figure 1 illustrates both the surgical and CPB preparations. Between experiments, all reusable components were gas sterilized with ethylene oxide to ensure sterility. The oxygenators were not reused.

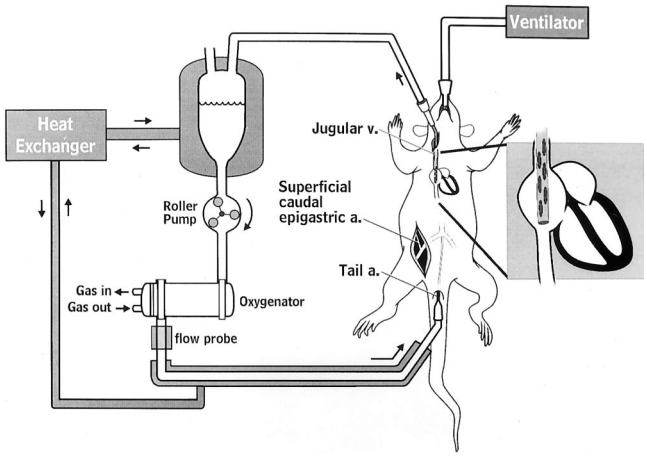


**Fig. 1. Schematic diagram of the surgical preparation and rat cardiopulmonary bypass apparatus. Modified from Grocott *et al.*[21] with permission.**

Rectal temperature was monitored and servo-regulated at 37.5 ± 0.1°C (YSI 400 series thermistor and 73ATA Indicating controller; YSI, Yellow Springs, OH) using a heating blanket and convective forced-air heating system. Mean arterial blood pressure was monitored *via* the superficial caudal epigastric artery, which was cannulated with polyethylene tubing (PE-10 Intramedic Tubing, Becton-Dickinson, Sparks, MD). Rats were given 150 IU heparin intravenously after placement of the arterial catheter. The tail artery was cannulated with a 20-gauge, 1.1-in intravenous catheter, which later served as the inflow for the CPB circuit. *Via* a neck incision, a 4.5-French, multiorifice, dual-stage venous cannula was inserted in the internal jugular vein and advanced until the tip of the cannula was placed near the junction of the inferior vena cava and right atrium. Position of the venous cannula had been confirmed in pilot experiments with the use of transesophageal echocardiography that used a commercially available intravascular ultrasound system. With the tip of the venous cannula in this position, drainage of the right atrium, the right superior vena cava, the left superior vena cava (found routinely in rats), and the inferior vena cava were optimized. Importantly, the rat has both left and right superior vena cavae, allowing the right to be ligated without adversely affecting cerebral venous outflow.

The CPB circuit consisted of a venous reservoir, a peristaltic pump, a membrane oxygenator, and the arterial inflow cannula, all of which were connected *via* 1.6-mm-ID silicone tubing (Tygon®; Cole-Parmer Instrument Co., Vernon Hills, IL). Blood returning through the venous cannula to the venous reservoir (jacketed with circulating water from a heat pump) was drained to a peristaltic pump (Masterflex®; Cole-Parmer Instrument Co.). Blood was pumped through a membrane oxygenator (a modified Cobe Micro® neonatal oxygenator with a surface area of 0.33 $m^2$; Cobe Cardiovascular, Inc., Arvada, CO) and then back into the animal *via* the arterial inflow cannula. An in-line flow probe (2N806 flow probe ands T208 volume flowmeter; Transonics Systems, Inc., Ithaca, NY) was used to measure CPB flow continuously. The CPB flow was 160–180 ml · $kg^{-1}$ · $min^{-1}$, which is similar to the normal cardiac output in the rat.[17,18] Arterial line inflow temperature was maintained at 37.5°C using a circulating water bath system. Complete CPB was assumed by (1) the absence of pulsatility in the monitoring arterial line; (2) transesophageal echocardiographic demonstration of effective drainage of the right atrium resulting in empty right and left ventricles; (3) pilot experiments that directly visualized (*via* thoracotomy) the collapse of the right atrium around the venous cannula with onset of CPB; and (4) complete dependence on pump flow for the maintenance of blood pressure seen during pilot experiments and abrupt cessation of the pump output.

The CPB circuit was primed with approximately 40 ml whole blood obtained before the start of the experiment from two heparinized (100 IU per rat, intravenous) donor rats, which were exsanguinated during isoflurane anesthesia. In addition, 6% hetastarch (3–5 ml) was added to the circuit during CPB to maintain adequate concentrations in the venous reservoir. While excessive hemodilution was avoided by priming the circuit with whole blood, the resulting hemoglobin concentration range was approximately 10–13 mg/dl. No further blood transfusion was required. Sham-operated animals received no additional intravenous fluid. Neither group received any exogenous glucose. The venous oxygen saturation from the venous return line was measured continuously using an Oximetrix® Monitor and Opticath® Catheter (Abbot Laboratories, North Chicago, IL). Arterial blood gas analysis was performed using an IL 1306 blood gas analyzer (Instrument Laboratories, Inc., Lexington, MA), with hemoglobin determined using an OSM3 Hemoximeter® (Radiometer Inc., Copenhagen, Denmark), both of which were calibrated to rat blood.

After the surgical preparation, local anesthetic was instilled into the wounds, which were closed around the catheters. After priming of the CPB circuit, baseline physiologic measurements were performed, and 10 min before commencement of CPB, the anesthetic was converted from isoflurane to fentanyl (150 μg/kg, intravenous), diazepam (2 mg/kg, intravenous), and pancuronium (0.2 mg, intravenous). A subsequent dose of fentanyl (75 μg/kg, intravenous) and diazepam (1 mg/kg, intravenous) was given after 30 min of CPB, thereby insuring an adequate depth of anesthesia. Pilot studies had been performed to assure that rats would not exhibit an escape response in the absence of pancuronium given this anesthetic regimen.

All animals underwent the same surgical preparation and were exposed to the same anesthetic conditions as described. The rats were then randomly assigned to receive either CPB (n = 10) or to serve as sham-operated

controls (n = 10). In the CPB group, CPB was instituted and maintained for 60 min. To prevent atelectasis during CPB, the lungs were not ventilated but received 5 mmHg of continuous positive airway pressure (fraction of inspired oxygen [$F_{IO_2}$] = 0.21). After 60 min of CPB, the animals were weaned from CPB without the need for inotropes or vasopressors. The heparin anticoagulation was allowed to dissipate spontaneously without supplemental administration of protamine. After decannulation, rats remained anesthetized, intubated, and ventilated for 2 h, after which any residual neuromuscular blockade was reversed with neostigmine (50 μg/kg, intravenous) and glycopyrrolate (20 μg/kg, intravenous). When the animals resumed spontaneous ventilation, the tracheas were extubated. The animals were allowed to recover in an oxygen-enriched environment for 24 h with free access to water and food. The control group had all CPB catheters left in place for the 60 min (thereby mimicking the surgical procedures for CPB) but were not connected to the external CPB circuit itself. The animals were decannulated and recovered in the same fashion as the CPB group did.

All animals underwent standardized functional neurologic testing. Twenty-four hours after CPB–sham operation, all animals underwent testing according to an established protocol that included assays of prehensile traction, strength, and balance-beam performance, which was graded on a 0–9 scale (best score = 9).[13,15] This testing was repeated again on the 3rd and 12th postoperative days.

In addition to the neurologic evaluation, behavioral testing using the Morris water maze to evaluate neurocognitive outcome was instituted on the third postoperative day by an investigator blinded to group assignment.[12] Briefly, the Morris water maze consisted of a 1.5-m-diameter, 30-cm-deep pool of water (27°C) with a submerged (1 cm below surface), hidden platform in one quadrant. Rats were placed in the water in a dimly lit room with multiple extra maze visual clues. The time to locate the submerged platform (defined as the latency) was measured to test for of impairment in visual–spatial learning and memory. Rats underwent daily testing in the water maze with four trials per testing period, each limited to a 90-s water exposure. Each of the trials was begun from a separate quadrant. Testing was performed daily until the 12th postoperative day (10 consecutive days of testing). To ensure that a difference in swimming speed between CPB animals and sham-operated controls would not confound the water maze results, the final nine rats (CPB, n = 5; sham-operated controls, n = 4) had their swimming recorded by a computerized video tracking system (EthoVision®; Noldus, Wageningen, The Netherlands).

After completion of the neurologic testing on the final day, the animals were anesthetized in 3% halothane and underwent *in situ* brain fixation using an intracardiac injection of 4% buffered formalin. Twenty-four hours later, the brains were removed and stored in 4% formalin. Paraffin-embedded brain sections were then serially cut (5-μm-thick sections) and stained with acid fuchsin–celestine blue. Images of the brain were then evaluated by light microscopy by one of the investigators (Y. S.) as well as a neuropathologist. With these investigators blinded to group assignment, the hippocampus was examined for evidence of neuronal necrosis (eosinophilic and pyknotic cells). The total number of both normal and necrotic cells were recorded. In addition, representative sections of the cerebellum, cortex, and caudoputamen were also stained, examined, and qualitatively assessed for the presence of necrotic injury.

### *Statistical Analysis*

Water maze performance, the primary outcome, was compared between groups using repeated-measures analysis of variance. Physiologic values were compared between groups using the Student *t* test, with the Bonferroni correction used to control for multiple comparisons. Neurologic outcome between groups on post-CPB days 1, 3, and 12 was compared using repeated-measures analysis of variance. Between-group comparisons of the average swimming speed over the 10-day swimming period were performed using the Student *t* test. Histologic outcomes were compared between groups using the Mann–Whitney U test. Statistical significance was assumed when $P < 0.05$.

## Results

Table 1 displays physiologic values from the CPB and sham-operated rats. The only differences between groups were in regard to a lower hemoglobin and a transient higher arterial oxygen tension ($Pa_{O_2}$) in the CPB *versus* the sham-operated group, both due to the nature of CPB. There were several deaths in both groups, both due to technical limitations (unintentional disconnection of the CPB circuitry; one CPB animal), insufficient venous return (one CPB animal), perforation of the right atrium (one sham-operated animal), and failure to resume normal eating and drinking patterns postoperatively (days 4–6, n = 3 CPB group, none in the sham-operated group). These animals were all excluded from the analysis, leaving a total of 10 animals in each group for analysis.

The CPB group had a worse neurologic outcome compared with the sham-operated controls on the 1st, 3rd, and 12th postoperative days ($P < 0.001$). Qualitative analysis of the individual components within the functional neurologic testing suggested that this difference was predominantly attributable to worse performance

**Table 1. Physiologic Values**

| | CPB | | | After CPB |
|---|---|---|---|---|
| | Baseline | 30 min | 60 min | 60 min |
| MAP (mmHg) | | | | |
| CPB | 83 (12) | 74 (14) | 78 (11) | 95 (8) |
| Control | 89 (8) | 82 (18) | 88 (8) | 91 (11) |
| CPB flow (ml · kg$^{-1}$ · min$^{-1}$) | | | | |
| CPB | | 165 (14) | 161 (15) | |
| Control | | — | — | |
| Hemoglobin (mg/dl) | | | | |
| CPB | 13.9 (1.3) | 10.9 (0.9) | 10.5 (0.7) | 10.8 (0.5) |
| Control | 13.6 (0.4) | 13.3 (0.7)* | 12.9 (0.9)* | 12.3 (0.6)* |
| Glucose (mg/dl) | | | | |
| CPB | 125 (22) | 127 (19) | 135 (23) | 136 (24) |
| Control | 120 (12) | 110 (5) | 128 (8) | 126 (6) |
| $Sa_{O_2}$ (%) | | | | |
| CPB | 98 (0.9) | 99 (0.7) | 99 (0.9) | 98 (2) |
| Control | 96 (0.6) | 98 (0.7) | 98 (0.6) | 97 (0.7) |
| $S\bar{v}o_2$ (%)† | | | | |
| CPB | | 55 (6) | 57 (8) | |
| Control | | — | — | |
| *p*Ha | | | | |
| CPB | 7.41 (0.04) | 7.40 (0.06) | 7.35 (0.09) | 7.35 (0.07) |
| Control | 7.40 (0.03) | 7.36 (0.03) | 7.36 (0.03) | 7.34 (0.04) |
| $Pa_{O_2}$ (mmHg) | | | | |
| CPB | 183 (56) | 436 (31) | 422 (38) | 201 (42) |
| Control | 171 (25) | 191 (26)* | 208 (19)* | 209 (31) |
| $Pa_{CO_2}$ (mmHg) | | | | |
| CPB | 39 (6) | 35 (4) | 34 (9) | 39 (5) |
| Control | 37 (5) | 37 (5) | 35 (4) | 38 (2) |
| $HCO_3^-$ (mEq) | | | | |
| CPB | 22.5 (1.2) | 23.5 (1.4) | 23.7 (2.1) | 22.3 (1.1) |
| Control | 23.0 (1.5) | 22.3 (1.8) | 23.1 (1.1) | 23.7 (0.8) |
| Rectal temperature (°C) | | | | |
| CPB | 37.3 (0.3) | 37.4 (0.1) | 37.5 (0.5) | 37.5 (0.2) |
| Control | 37.4 (0.2) | 37.4 (0.1) | 37.5 (0.2) | 37.4 (0.4) |

Values are mean (SD). n = 10, CPB; n = 10, sham-operated control.

* $P < 0.05$. † $S\bar{v}o_2$ was performed in the outlet from the venous reservoir so was unavailable in the control group.

CPB = cardiopulmonary bypass; MAP = mean arterial pressure; $Sa_{O_2}$ = arterial oxygen saturation; $S\bar{v}o_2$ = mixed venous oxygen saturation; *p*Ha = arterial *p*H; $Pa_{O_2}$ = arterial oxygen tension; $Pa_{CO_2}$ = arterial carbon dioxide tension; $HCO_3$ = bicarbonate.

on the balance beam and prehensile traction ability (table 2).

Figure 2 represents the Morris water maze performance in the two groups. CPB rats showed longer water maze latencies compared with the sham-operated controls ($P = 0.004$), indicating significant neurocognitive dysfunction after CPB. Figure 3 represents the daily water maze swimming speed of the rats. The average swimming speed (days 3–12) was not different between groups (CPB group, 27.2 ± 2.8 cm/s; sham-operated group, 25.3 ± 2.7 cm/s; $P = 0.34$).

Despite these marked differences in functional neurologic and neurocognitive outcome, histologic outcome between the two groups was less clear. There was no difference between groups with respect to total number of hippocampal neurons or total number of necrotic hippocampal neurons (table 3). Assessment of representative sections of the cerebellum, cortex, and caudoputamen showed no evidence of gross neuronal injury.

## Discussion

Although acute models of partial CPB in the rat have been reported previously,[19,20] the current study represents the primary description of long-term recovery after complete CPB in the rat. Rats undergoing CPB demonstrated both neurologic and neurocognitive impairment when compared with sham-operated controls.

The current study extends our earlier work in which we first demonstrated the feasibility of complete CPB followed by short-term (24-h) recovery in the rat.[21] In the current study, we allowed a recovery interval of 12 days, which gave us the opportunity to examine neurologic, neurocognitive, and histologic outcomes. The ability to obtain longer-term recovery from CPB is an essential condition if lasting effects of CPB on various organ systems are studied. In particular, delayed neuronal necrotic (although not present herein) and apoptotic mechanisms may dramatically affect the extent of mea-

**Table 2. Neurologic Outcome**

| | Test | Control | CPB |
|---|---|---|---|
| 24 h | Screen | 3.0 ± 0 | 3.0 ± 0 |
| | Balance | 3.0 ± 0 | 1.6 ± 1.1 |
| | Traction | 2.6 ± 0.5 | 1.8 ± 0.6 |
| | Total | 8.6 ± 0.5 | 6.4 ± 1.4 |
| 72 h | Screen | 3.0 ± 0 | 3.0 ± 0 |
| | Balance | 3.0 ± 0 | 2.0 ± 0.8 |
| | Traction | 3.0 ± 0 | 2.0 ± 0.7 |
| | Total | 9.0 ± 0 | 7.0 ± 0.9 |
| 12 days | Screen | 3.0 ± 0 | 2.8 ± 0.4 |
| | Balance | 3.0 ± 0 | 1.6 ± 0.8 |
| | Traction | 2.7 ± 0.5 | 1.6 ± 0.7 |
| | Total | 8.7 ± 0.5 | 6.0 ± 1.2 |

Neurologic outcome as assessed with the neuromotor scoring system represented by three different assays: rotating screen (screen), balance beamperformance (balance), and prehensile traction (traction). Values are mean ± SD. A score of 3 in any category indicates normal performance. The cardiopulmonary bypass (CPB) group had a worse neuromotor score compared with the sham-operated control group ($P = 0.0001$).

surable neurologic injury in models of cerebral injury.[22–24] Without adequate time for degenerative and reparative mechanisms to occur, at least some of the effects of CPB may be missed.

In a recent editorial, Hindman *et al.*[6] stated that there is a lack of a suitable animal model of CPB by which to study both the mechanisms behind CPB-related neurologic injury and experimental compounds that potentially could alter cerebral outcomes after cardiac surgery using CPB. A number of large animal models of CPB in pigs, dogs, and rabbits have been described.[7–9] All had limitations with respect to costs of equipment and animals, technical difficulties associated with performing CPB in larger animals, and limited recovery periods due

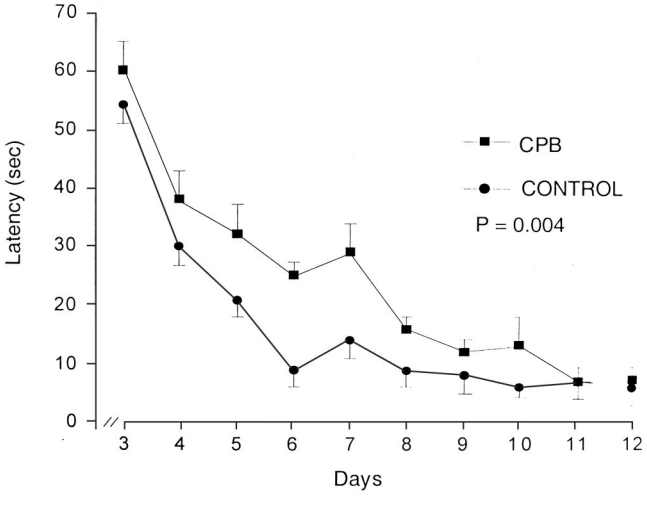


**Fig. 2. Neurocognitive outcome as assessed daily from postoperative days 3–12 after cardiopulmonary bypass (CPB) by visual–spatial learning with the Morris water maze. Analyzing the group mean latency in a repeated measures of analysis of variance, the CPB group had longer latencies compared with the sham-operated control group ($P = 0.004$), indicating significant neurocognitive dysfunction after CPB.**

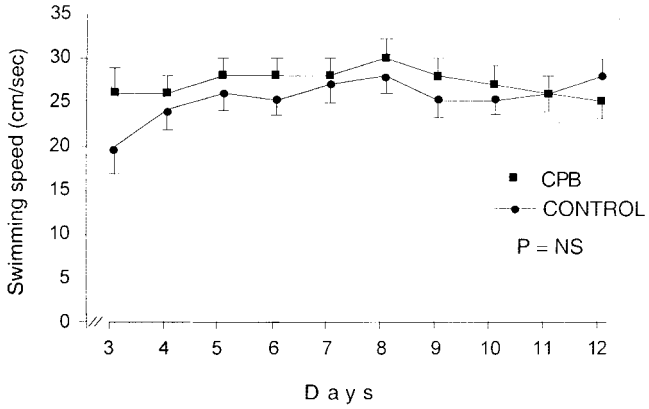


**Fig. 3. Water maze swimming speed as assessed using a computerized video tracking system in a subset of rats (cardiopulmonary bypass [CPB], n = 5; sham operated, n = 4). The average swimming speed (days 3–12) was not different between groups ($P = 0.34$). NS = not significant.**

to significant cost, morbidity, and mortality associated with recovery after CPB. The rat offers several advantages, being a species in which the effects of cerebral ischemia on neuronal and neurologic outcome have been extensively studied and characterized.[10,14,15] A recent literature review by Ballaux *et al.*[20] outlining the need for and advantages of a preclinical model of CPB in small animals describes a number of earlier attempts to develop a model of CPB in the rat. According to those authors, the advantages include the reduction of costs of animals and equipment as well as the fact that a full-scale operating environment is not needed. Existing rat models have achieved only partial CPB or, because of invasive cannulation (usually with sternotomy), have made long-term survival impossible. Open-chest surgery severely limits recovery in small rodents, partly because of the need for intact sternal musculature for ambulation. Further, because several of these earlier descriptions of CPB in the rat are more than two decades old,[25–27] recent technical developments and improvements to the CPB circuitry are not reflected, which makes these older models less relevant to the current clinical situation. This, and the opportunity to develop a technically feasible and relatively inexpensive recovery model, prompted us to pursue new attempts to develop a long-term recovery model of complete CPB in the rat.

**Table 3. Histology Results**

| | Normal-appearing Cells | | Dead Cells | |
|---|---|---|---|---|
| | CA 1–2 | CA 3 | CA 1–2 | CA 3 |
| CPB | 490 ± 85 | 186 ± 112 | 9 ± 5 | 3 ± 2 |
| Control | 501 ± 99 | 205 ± 124 | 10 ± 5 | 2 ± 3 |
| *P* | 0.48 | 0.68 | 0.86 | 0.59 |

Values are mean ± SD. Cell counts (normal and dead appearing cells) in the hippocampal segments CA 1–2 and CA 3. Cardiopulmonary bypass (CPB): n = 10; control: n = 10. No difference was present between both groups with respect to total number of hippocampal neurons or total number of necrotic hippocampal neurons.

However, there are some limitations to this current model. Although our model closely resembles current clinical standards with respect to the CPB circuit, a number of potentially important differences to the clinical setting are present. Median sternotomy, direct surgery on the heart involving aortic cross-clamping, and cardiac arrest with the use of cardioplegia were not performed. Similarly, the absence of significant atheromatous disease and the complex comorbidities seen in patients undergoing coronary artery bypass graft surgery are limitations. All these factors are thought to contribute, at least in part, to cerebral injury after cardiac surgery. With respect to our aortic cannulation site, the retrograde aortic perfusion used in our model is not as commonly used as antegrade perfusion; however, it has been used extensively for PortAccess (Heartport Inc., Redwood City, CA) minimal access cardiac surgery and is frequently used for operations on the intrathoracic aorta using CPB.

Our finding of a discordance between functional outcome and histologic damage warrants special consideration. There are several possible explanations for this discrepancy. With respect to the neurologic outcome, given the absence of any neuronal necrosis in either CPB or sham-operated animals, the relatively high scores in the strength-related parts of the neuromotor function test are not surprising.[13] Previous work has shown that this neuromotor scoring system, although commonly used in rat models, is not sufficiently sensitive to detect behavioral differences, even after substantial ischemic damage subsequent to experimental ischemia.[13,15] Therefore, we included a more sensitive assay of cerebral dysfunction (the Morris water maze test) in the current study. There was again no apparent relation between this measure of neurocognitive outcome and histology. A potential explanation for this might relate to the dependence of swimming on motor strength and function. That is, if the animals were physically weaker in the CPB group, they may not have been able to swim properly, which might artificially increase their latency times. However, this is unlikely because the swimming speeds measured were similar between groups, actually tending to be slightly better in the CPB group (fig. 3).

The absence of a histologic correlate could also be due to our choice of histologic technique as well as the time point chosen. The stains chosen were targeted for necrotic neurons.[22,28,29] There may have been apoptotic neurons that we were unable to image. Neuronal necrosis is believed to occur more rapidly, whereas apoptosis may occur over longer time periods. However, by day 12, both of these processes should be well underway (apoptosis) or completed (necrosis). If necrosis, apoptosis, or both had occurred, we would have expected to see a reduced total number of normal neurons, so this seems an unlikely explanation. However, the most likely reason for our finding may relate to direct neuronal dysfunction in the absence of actual cell death that might then lead to the functional outcome differences that we measured. Interestingly, impairments of visual–spatial learning, as seen in the current study, have been associated with distinct changes in synaptic plasticity of the hippocampus in diabetic rodents.[30] Cognitive ability depends on the synaptic integrity of the hippocampus.[31,32] Changes of synaptic plasticity in CA1 pyramidal cells of the hippocampus have also been reported in models of cerebral ischemia in rats and can be detected long before cell death.[33,34] To our knowledge, the role of hippocampal synaptic plasticity in the context of neurocognitive dysfunction after CPB has not been investigated.

The potential ability of this model to investigate cerebral injury after cardiac surgery relates to its ability to address mechanistic questions as well as neuroprotective strategies. The exact etiology of CPB-associated neurologic injury is unknown, but it is likely that a number of contributing factors have a role. Several potential factors can be investigated with our model, including systemic inflammation,[35–37] genetic influences (*e.g.*, apolipoprotein genotype),[38] apoptosis,[39,40] cerebral edema,[41] and hyperthermia during rewarming or after separation from cardiopulmonary bypass[42,43]. Inflammatory cascades may be particularly important in CPB-associated cerebral injury because they are involved not only as a result of cerebral ischemia (assumed to be secondary to embolization and global hypoperfusion) but also as part of the "whole body" inflammatory response initiated by the contact of blood with foreign surfaces in the CPB apparatus. In a recent study, Hindman *et al.*[44] demonstrated that in a similar but acute model of CPB in rats, the systemic inflammatory response was mild and delayed but was represented by increasing brain cyclooxygenase (COX2) messenger RNA expression at 4 h after CPB. They speculated that COX2 induction may alter cerebral physiology after CPB.[45]

In summary, this study shows the presence of both neurologic and neurocognitive impairment in a rodent recovery model of CPB similar to that commonly observed in humans subjected to a similar procedure. Because of the availability of sophisticated methods of neuroscientific inquiry into mechanisms of injury and therapy for this species, use of this CPB model may allow advance in our understanding of the clinical neurocognitive injury associated with CPB.

The authors thank Betty Smith and Ann Brinkhous (Duke University Multidisciplinary Neuroprotection Laboratories) for their expert technical assistance with the conduct of the experiments and Dr. C. Hullete, MD, (Duke University, Department of Pathology) for reviewing the histopathologic sections.

## References

1. Gardner T, Horneffer P, Manolio T, Pearson T: Stroke following coronary bypass grafting: A ten year study. Ann Thorac Surg 1985; 40:574–81

2. Tuman KJ, McCarthy RJ, Najafi H, Ivankovich AD: Differential effects of advanced age on neurologic and cardiac risks of coronary artery operations. J Thorac Cardiovasc Surg 1992; 104:1510–7

3. Newman M, Wolman R, Kanchuger M, Marschall K, Mora-Mangano C, Roach G, Smith LR, Aggarwal A, Nussmeier N, Herskowitz A, Mangano DT: Multicenter preoperative stroke risk index for patients undergoing coronary artery bypass surgery. Multicenter Study of Perioperative Ischemia (McSPI) Research Group. Circulation 1996; 94:II74–80

4. Roach G, Kanchuger M, Mora Mangano C, Newman M: Adverse cerebral outcomes after coronary bypass surgery. N Engl J Med 1996; 335:1857–63

5. Newman M, Kirchner J, Phillips-Bute B, Gaverm V, Grocott H, Jones R, Mark D, Reves J, Blumenthal J: Longitudinal assessment of neurocognitive function after cardiac surgery: Perioperative decline predicts long-term (5-year) neurocognitive deterioration. N Engl J Med 2001; 344:395–402

6. Hindman BJ, Todd MM: Improving neurologic outcome after cardiac surgery. ANESTHESIOLOGY 1999; 90:1243–7

7. Hindman BJ, Funatsu N, Harrington J, Cutkomp J, Dexter F, Todd MM, Tinker JH: Cerebral blood flow response to $Pa_{CO_2}$ during hypothermic cardiopulmonary bypass in rabbits. ANESTHESIOLOGY 1991; 75:662–8

8. Johnston WE, Stump DA, DeWitt DS, Vinten-Johansen J, O'Steen WK, James RL, Prough DS: Significance of gaseous microemboli in the cerebral circulation during cardiopulmonary bypass in dogs. Circulation 1993; 88:II319–29

9. Bokesch PM, Seirafi PA, Warner KG, Marchand JE, Kream RM, Trapp B: Differential immediate–early gene expression in ovine brain after cardiopulmonary bypass and hypothermic circulatory arrest. ANESTHESIOLOGY 1998; 89:961–8

10. Smith ML, Bendek G, Dahlgren N, Rosen I, Wieloch T, Siesjö BK: Models for studying long-term recovery following forebrain ischemia in the rat, 2: A 2-vessel occlusion model. Acta Neurol Scand 1984; 69:385–401

11. Garcia JH, Wagner S, Liu KF, Hu XJ: Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats: Statistical validation. Stroke 1995; 26:627–34

12. Morris R: Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11:47–60

13. Combs D, D'Alecy L: Motor performance in rats exposed to severe forebrain ischemia: Effect of fasting and 1,3-butanediol. Stroke 1987; 18:503–11

14. Ginsberg MD, Busto R: Rodent models of cerebral ischemia. Stroke 1989; 20:1627–42

15. Gionet T, Thomas J, Warner D, Goodlett C, Wasserman E, West J: Forebrain ischemia induces selective behavioral impairments associated with hippocampal injury in rats. Stroke 1991; 22:1040–7

16. Guide for the Care and Use of Laboratory Animals, publication No. 86-23. Bethesda, Health and Human Services, National Institutes of Health, revised 1996

17. Chenitz W, Nevins B, Hollenberg N: Preglomerular resistance and glomerular perfusion in the rat and dog. Am J Physiol 1976; 231:961–6

18. Li SG, Randall DC, Brown DR: Roles of cardiac output and peripheral resistance in mediating blood pressure response to stress in rats. Am J Physiol 1998; 274:R1065–9

19. Mendler N, Weishaar E, Brendel W: Eine herz-lungen-maschine fur ratten als experimentelles modell der extracorporeal zirkulation. Thoraxchirurgie 1969; 17:534–8

20. Ballaux PK, Gourlay T, Ratnatunga CP, Taylor KM: A literature review of cardiopulmonary bypass models for rats. Perfusion 1999; 14:411–7

21. Grocott H, Mackensen G: A recovery model of cardiopulmonary bypass in the rat. Perfusion 2001; 16:75–81

22. Kirino T, Tamura A, Sano K: Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. Acta Neuropathol 1984; 64:139–47

23. Vaux DL, Strasser A: The molecular biology of apoptosis. Proc Natl Acad Sci U S A 1996; 93:2239–44

24. Choi D: Ischemia-induced neuronal apoptosis. Curr Opin Neurobiol 1996; 6:667–72

25. Subramanian V, McLeod J, Gans H: Effect of extracorporeal circulation on reticuloendothelial function, I: Experimental evidence for impaired reticuloendothelial function following cardiopulmonary bypass in rats. Surgery 1968; 64: 775–84

26. Mendler N, Reulen H, Brendel W: Cold swelling and energy metabolism in the hypothermic brain of rats and dogs, Hibernation and Hypothermia: Perspective and Challenges. Edited by South F, Hannon J, Willis J, Pengelley E, Alpert N. Amsterdam, Elsevier, 1972, pp 167–90

27. Proctor E: An oxygenator for cardiopulmonary bypass in the rat. J Surg Res 1977; 22:124–7

28. Nakano S, Kogure K, Fujikura H: Ischemia-induced slowly progressive neuronal damage in the rat brain. Neuroscience 1990; 38:115–24

29. Kawaguchi M, Kimbro JR, Drummond JC, Cole DJ, Kelly PJ, Patel PM: Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. ANESTHESIOLOGY 2000; 92:1335–42

30. Gispen WH, Biessels GJ: Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci 2000; 23:542–9

31. Morris RG, Garrud P, Rawlins JN, O'Keefe J: Place navigation impaired in rats with hippocampal lesions. Nature 1982; 297:681–3

32. Jarrard LE: What does the hippocampus really do? Behav Brain Res 1995; 71:1–10

33. Miyazaki S, Katayama Y, Furuichi M, Kinoshita K, Kawamata T, Tsubokawa T: Impairment of hippocampal long-term potentiation following transient cerebral ischaemia in rat: Effects of bifemelane, a potent inhibitor of ischaemia-induced acetylcholine release. Neurol Res 1993; 15:249–52

34. Aoyagi A, Saito H, Abe K, Nishiyama N: Early impairment and late recovery of synaptic transmission in the rat dentate gyrus following transient forebrain ischemia in vivo. Brain Res 1998; 799:130–7

35. Butler J, Rocker GM, Westaby S: Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 1993; 55:552–9

36. Hall RI, Smith MS, Rocker G: The systemic inflammatory response to cardiopulmonary bypass: Pathophysiological, therapeutic, and pharmacological considerations. Anesth Analg 1997; 85:766–82

37. Murkin J: Cardiopulmonary bypass and the inflammatory response: A role for serine protease inhibitors? J Cardiothorac Vasc Anesth 1997; 11:19–23

38. Newman, Laskowitz DT, Saunders AF, Grigore AM, Grocott HP: Genetic predictors of perioperative neurologic and neuropsychological injury and recovery. Sem Cardiothorac Vasc Anesth 1999; 3:34–46

39. Kurth CD, Priestley M, Golden J, McCann J, Raghupathi R: Regional patterns of neuronal death after deep hypothermic circulatory arrest in newborn pigs. J Thorac Cardiovasc Surg 1999; 118:1068–77

40. Sato, Laskowitz DT, Warner DS, Newman MF, Grocott HP: Cardiopulmonary bypass induces cerebral apoptotic gene expression in the rat. (abstract). Anesth Analg 2001; 92:S38

41. Harris D, Bailey S, Smith P, Taylor K, Oatridge A, Bydder G: Brain Swelling in first hour after coronary artery bypass surgery. Lancet 1993; 342:586–7

42. Cook D, Orszulak T, Daly R, Buda D: Cerebral hyperthermia during cardiopulmonary bypass in adults. J Thorac Cardiovasc Surg 1996; 111:268–9

43. Grocott H, Croughwell N, Lowry E, White W, Newman M, Reves J: Continuous jugular venous versus nasopharyngeal temperature monitoring during hypothermic cardiopulmonary bypass for cardiac surgery. J Clin Anesth 1997; 9:312–6

44. Hindman B, Moore S, Cutkomp J, Smith T: Cardiopulmonary bypass (CPB) increases brain inducible cyclooxygenase (COX2) mRNA expression in rats (abstract). ANESTHESIOLOGY 2000; 93:A695

45. Rivest S: What is the cellular source of prostaglandins in the brain in response to systemic inflammation? Facts and controversies. Mol Psychiatry 1999; 4:500–76