


# Animal Models of Cardiopulmonary Bypass: Development, Applications, and Impact

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## Abstract

Neurologic and neurocognitive complications after cardiac surgery have been reported repeatedly. To better understand its etiology and design protective strategies, small animal models have been developed. This study describes the development of a survival rat cardiopulmonary bypass (CPB) model, along with the introduction of an appropriately sized oxygenator. This model led the way for even more complicated models with CPB, facilitating full cardiac arrest with antegrade cardioplegia administration, air embolization, and deep hypothermic circulatory arrest. In addition, the results of several of those rat CPB studies are summarized and their preclinical relevance is pointed out.

## Keywords

animal model, cardiopulmonary bypass, inflammation, deep hypothermic circulatory arrest, rat, embolization

Postoperative neurologic deficits following cardiac surgery using cardiopulmonary bypass (CPB) present common and severe complications. These deficits can range from devastating injuries, such as stroke, to more subtle neurocognitive dysfunction.<sup>1,2</sup> The mechanisms leading to cerebral injury are not fully understood and neuroprotective drugs or strategies have limited effects. To further elucidate the etiology of cerebral injury following cardiac surgery, an appropriate nonclinical disease model is needed. Such a disease model is also required to screen potential neuroprotective drugs in a preclinical environment with regard to safety concerns and effectiveness.

## Animal Models of Cardiopulmonary Bypass

Cerebral microembolization, hypoperfusion, cerebral edema, inflammation, hyperthermia, genetic disposition, and blood–brain barrier dysfunction have all been suggested as contributing factors to the etiology of neurologic and cognitive impairment after cardiac surgery.<sup>3–6</sup> In the ideal situation, an animal model serves to investigate one potentially contributing factor, whereas the others remain unchanged. To investigate the effect of embolization and hypoperfusion on the brain, several models have been developed.<sup>7–9</sup> However, the means by which CPB modifies the influence of each of these factors on neurologic outcomes is not understood. Thus, an animal model of CPB is required,

to facilitate research into the effects of CPB in relation to different insults, such as cerebral emboli, hypoperfusion, and hyperthermia. Moreover, the effect of various comorbidities, such as diabetes, age, or atherosclerosis, can then be investigated. Once a model is established, many potential neuroprotective drugs or strategies can be tested for effectiveness and tolerability in this appropriate animal model before it is investigated in humans.

To study neurologic outcomes following CPB, an ideal animal model needs to (a) allow long-term survival, (b) be technically simple so it can be performed ideally by one person, (c) be relatively inexpensive so that group sizes can be sufficient, (d) mirror clinical standards as closely as possible, and (e) use established and validated learning tasks to assess gross neurologic as well as neurocognitive outcomes.

Large animal models of CPB in dogs, lambs, pigs, and rabbits have been used but are limited by the costs of operating in a full scale environment and the personnel required.<sup>10–12</sup> Furthermore, tests to assess cognitive performance are not validated yet. In contrast, in the field of neurosciences, the

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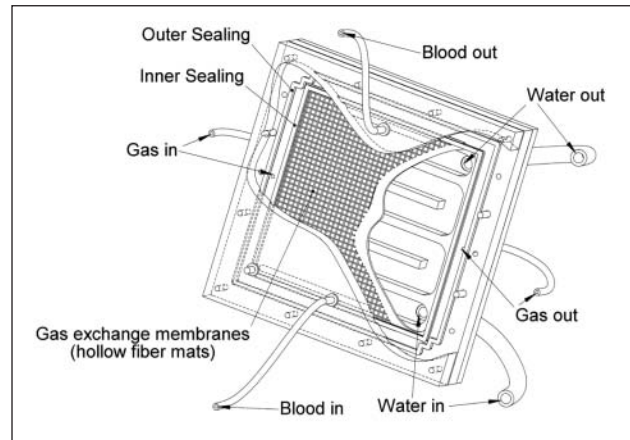
rat is a well-studied species with validated learning tasks available. In addition, in most situations a single experimenter can perform all research tasks. Therefore, a rat model of CPB would allow investigations of larger sample sizes and the assessment of long-term neurologic outcomes.

### Rat Models of Cardiopulmonary Bypass

To our knowledge, the first description of rats subjected to extracorporeal circulation was in 1967 from Popovic et al.<sup>13</sup> In the decades that followed, several other CPB models were developed, and these were designed to answer different research questions.<sup>14-16</sup> Most of them used bubble-oxygenators and full sternotomy and long-term survival was not a goal or not reported. For a detailed description of the older models, we refer to the review by Ballaux et al.<sup>17</sup> The rat models of CPB used so far confronted two problems: first, the rat does not easily survive sternotomy; and, second, venous drainage via peripherally inserted catheters (to avoid sternotomy) was not optimal with the consequence that only partial CPB was accomplished.<sup>14,18-20</sup> An important step forward was the introduction of a rat model that avoided sternotomy, but allowed for the conduct of complete CPB by optimizing venous drainage.<sup>21,22</sup> Using this model, Mackensen et al.<sup>21</sup> demonstrated neurocognitive deficits during the first 12 postoperative days using the Morris water maze. We studied the impact of CPB on long-term cognitive outcome in young and healthy rats and did not detect any difference between the animals exposed to CPB and the sham-operated animals.<sup>23-25</sup> Consequently, we exposed old and diabetic rats to CPB and assessed the short-term cognitive outcome with the Morris water maze; again, there was no deficit in the CPB groups.<sup>26</sup> Taken together, these results suggest that CPB alone does not lead to reproducible neurologic deficits, which is concordant with clinical trials comparing on-pump with off-pump procedures.<sup>27,28</sup> However, this model of CPB in rats presents an important starting point, as it permits the incorporation of further experimental insults and risk factors, such as emboli, hypoperfusion, comorbidities, and age into the setting of CPB.

### Further Development and Applications of Cardiopulmonary Bypass Models in Rats

Most of the models described above used oversized gas exchange devices ("oxygenators") compared with the size of the rat. Frequently, the smallest clinically available neonatal oxygenators were used.<sup>19,21,23</sup> The consequence of these large priming volumes is that usually 2 blood donor rats were required to prime the circuit with blood. As this practice



**Figure 1.** Three-dimensional drawing of the oxygenator specifically designed for the use in rats

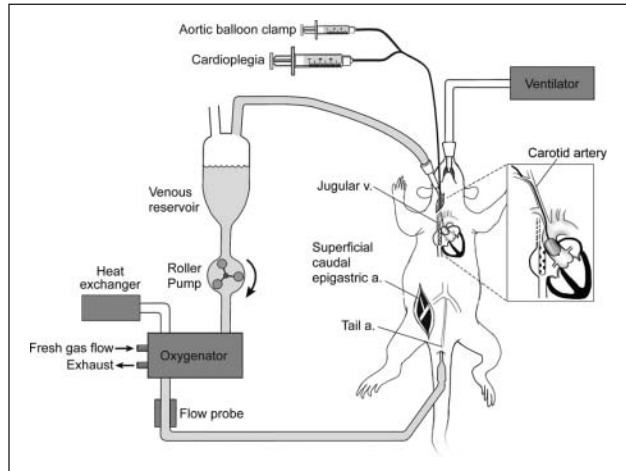
introduces error and increases variability in several outcome parameters, an appropriately sized circuit with oxygenator was needed. Gourlay et al.<sup>20</sup> described an oxygenator with a 4 mL priming volume and an overall circuit priming volume of 12 mL to study pulsatile CPB. We also introduced an oxygenator specifically designed for the use in rats: it consists of 2 Plexiglas shells supporting the diffusion membrane (Figure 1). The gas exchange area is 558 cm<sup>2</sup> and it allows sufficient oxygenation and CO<sub>2</sub> elimination for flow rates that match the normal cardiac output of awake rats. The oxygenator requires 4 mL for priming and the total circuit volume is 10 mL. Thus, the conduct of CPB without any donor blood is possible using this setup.<sup>29</sup> A study comparing the oversized neonatal oxygenator with one specifically designed for use in rats showed higher systemic IL-6 in the group with the neonatal oxygenator. Inflammatory reactions in the brain were pronounced in the CPB groups, compared with the sham-operated animals; oxygenator used had no impact. Surprisingly, a pronounced cerebral inflammation after CPB was not associated with any cognitive impairment.<sup>24</sup> This suggests that factors other than CPB and inflammatory responses might be contributing to adverse cognitive outcomes after cardiac surgery.<sup>30</sup>

As stated above, gaseous or particulate emboli are thought to be an important contributing factor to adverse cerebral outcomes following cardiac surgery.<sup>3,31</sup> Therefore, cerebral air embolization was incorporated into the improved CPB model in rats. Based on established models of cerebral air embolism in rodents,<sup>9</sup> the emboli were directly injected into the cerebral circulation to ensure standardized and controlled embolization. A dose-escalating study showed that significantly smaller volumes of cerebral air emboli were tolerated in the presence of CPB, suggesting an additive negative effect of CPB and emboli on the brain.<sup>32</sup>

Cerebral hypoperfusion is hypothesized to be another factor leading to neurologic dysfunction following cardiac

surgery.<sup>5</sup> The extreme variant of cerebral hypoperfusion is represented by circulatory arrest, which is performed as deep hypothermic circulatory arrest (DHCA) for the repair of congenital heart disease and for complex thoracic aortic surgery. Using the model of CPB in rats, we performed DHCA for different durations and assessed survival, motor function and histological outcome. The survival rate decreased dramatically after DHCA durations exceeding 60 minutes whereas the percentage of animals without any motor deficits after 14 days decreased to 50% following 45 minutes of DHCA.<sup>29</sup> Using 45 minutes of DHCA, we have shown an accentuated inflammation in the brain in addition to neurologic deficits on postoperative day 1.<sup>33</sup> With the incorporation of emboli and DHCA into the existing model of CPB, we have a disease model that leads to motor and cognitive dysfunction as well as histological alterations.

Even though the original purpose of the model was to assess neurocognitive outcomes, it has been used to test several different problems related to cardiac surgery. Perioperative cardiovascular complications are described in up to 25% of patients. These include prolonged myocardial contractile dysfunction (stunning), myocardial infarction, low-output syndromes, and overt ventricular failure—all of which are associated with prolonged intensive care unit stays, reduced functional capacity at discharge, and increased overall mortality.<sup>34,35</sup> The etiology of myocardial dysfunction following cardiac surgery is multifactorial, but frequently involves perioperative myocardial ischemia and reperfusion injury.<sup>36</sup> Since the advent of CPB, cardioplegic arrest has been an essential component of cardiac surgery, yet remains associated with myocardial ischemia–reperfusion injury. As surgical interventions become more complex, the resulting prolonged aortic cross-clamp and CPB times created the need for optimized myocardial protection strategies. Experimental efforts to better understand the underlying mechanisms associated with postoperative myocardial reperfusion injury and to improve established myocardial protection protocols have been limited to either costly large animal models or ex vivo heart preparations.<sup>37-39</sup> These models, however, do not facilitate research on the long-term effects of myocardial reperfusion injury or novel therapeutic interventions. Therefore, we adapted the model to induce complete cardiac arrest by administration of antegrade cardioplegia solutions and endoaortic clamping.<sup>40</sup> In this model, a balloon catheter is positioned just above the aortic valve (Figure 2). While on CPB, cardioplegia can be injected and the balloon inflated to serve as an endoaortic crossclamp. To rule out any gross neurological damage due to cannulation of the right carotid artery, a functional assessment and histological evaluation of the brains was performed.<sup>40</sup> Current research in this model now focuses on improved myocardial protection and genomics.



**Figure 2.** Schematic setup of cardiopulmonary bypass model with endoaortic cross-clamp and cardioplegia administration (arterial line via superficial caudal epigastric artery)

## Application of Animal Models for Preclinical Screening

It is clearly optimal to test potentially neuroprotective drugs' safety and effectiveness in animal models before experimental or use in humans should be pursued. In this context, we studied the safety aspects of the noble gas xenon in our combined model of CPB and cerebral air embolism. Xenon has been shown to be neuroprotective in several models of cerebral injury, including CPB.<sup>41,42</sup> However, xenon also possesses the capacity to expand intravascular air bubbles (that are introduced as cerebral air emboli during cardiac surgery). This could potentially abolish any neuroprotective effect or even amplify neurologic injury following CPB with cerebral air emboli.<sup>43,44</sup> Our study demonstrated worse fine-motor, cognitive, and histological outcomes in animals treated with xenon and exposed to CPB in the presence of cerebral air emboli, thereby fostering safety concerns.<sup>45</sup>

Another study was designed to examine the effects of the artificial oxygen carrier perfluorocarbon. We showed that administration of perfluorocarbon during CPB resulted in a remarkable inflammatory response, loss of vasomotor tone, and a very high mortality rate.<sup>46</sup> These experimental results confirmed the concerns raised during the conduct of a phase 3 trial, which was abandoned prematurely because of a higher incidence of neurologic complications in patients treated with perfluorocarbon.<sup>47</sup>

## Conclusion

In summary, patients undergoing cardiac surgery are at high risk for postoperative neurologic dysfunction. A long-term survival model of CPB in rats creates the potential to further

elucidate basic mechanisms leading to neurologic disorders. In this context, we have suggested that factors beyond CPB and inflammatory responses are likely contributing to the adverse neurocognitive outcomes observed following cardiac surgery. The further development of the rat CPB model into a DHCA model and a CPB with air embolization model has been important steps in optimizing this model as a screening tool for potential neuroprotective strategies. In this regard, we have shown that in the presence of CPB emboli are less well tolerated and that xenon amplifies the effect of air embolization in the setting of CPB.<sup>32,45</sup> Furthermore, we reported a very high mortality when perfluorocarbon was added to the CPB system as an artificial oxygen carrier.<sup>46</sup> In conclusion, a model of CPB in rats with incorporation of injurious events is suitable to not only improve our knowledge of underlying mechanisms but also to study the potential value of proposed neuroprotectants before advancing them into clinical trials.

### Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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