Persistent cognitive deficits and neuroinflammation in a rat model of cardiopulmonary bypass

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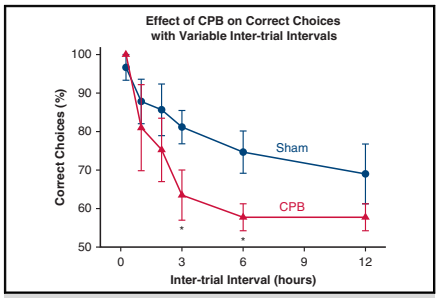
Advances in surgical techniques and medical management have substantially improved mortality and morbidity in patients undergoing cardiopulmonary bypass (CPB). Although postoperative cognitive dysfunction is common after CPB, the prevalence and mechanism(s) of intermediate and long-term cognitive deficits in the absence of neuronal loss remain a matter of discussion.[1] By using an established rat model of CPB[2] that does not display neuronal loss,[3,4] we examined behavioral and structural effects of CPB at 6 months postsurgery. Persistent deficits in performance on a complex behavioral task and sustained activation of macrophages/microglia were observed with no evidence of neuronal loss.



Performance on a cognitive task was impaired 6 months after CPB.

CENTRAL MESSAGE

Long-term cognitive dysfunction and neuroinflammation were demonstrated in an established rat model of CPB.

See Commentaries on pages e189 and e190.

MATERIALS AND METHODS

Male Sprague-Dawley rats (375-400 g) were randomized to 2 groups: (1) CPB and (2) sham surgery (n = 6/group). The CPB procedure, involving a 60-minute period of bypass, was performed as described previously[2] (Appendix E1). The sham surgery group received an identical surgical procedure and duration of anesthesia as did the CPB group, except that the CPB circuit was not activated. At 6 months post-CPB, behavioral testing was performed using a win-shift task on an 8-arm radial maze.[5] Win-shift is a challenging cognitive task involving a multistage foraging strategy that requires animals to retain and compare information about the location of food over a period of 0.25 to 12 hours. The task uses 2 sequential trials separated by a variable inter-trial interval. The first trial presents 4 food-baited arms in the maze with the other 4 arms blocked by a clear Plexiglass door. During the initial stage of task acquisition, the second (retention) trial occurs 5 minutes after the first trial. In the second trial, all maze arms are open, but only those arms that were not baited in the first trial are baited. A correct choice in the second trial is when an animal visits a baited (previously unbaited) arm of the maze. An incorrect choice is when an animal visits an unbaited (previously baited) arm. Once animals have acquired the task, the interval between the first and second trials is progressively increased in duration.

After behavioral testing was completed, animals were euthanized under deep anesthesia by intracardial perfusion of fixative. Brains were sectioned and processed for microscopic assessment of Nissl staining (Cresyl Violet), interneuron subtypes (anti-calretinin and anti-parvalbumin), immature neurons (anti-doublecortin), and macrophages/microglia (anti-CD68). Staining for degenerating neurons (Fluro-Jade) was performed in additional animals at 2, 4, and 7 days post-CPB (n = 2 animals/time point).

RESULTS

Animals in the CPB group exhibited deficits in performance on the win-shift task compared with those in the sham surgery group (Figure 1). At shorter inter-trial intervals, performance was similar between groups, indicating that animals in both groups were able to learn and perform the task. However, at longer inter-trial intervals (3 and 6 hours), the CPB group exhibited significantly poorer performance.

Qualitative assessments of Nissl-stained and immunohistochemically stained sections did not indicate a loss of primary neurons or interneurons in the hippocampus at 6 months post-CPB (Figure 2). In addition, Fluro-Jade

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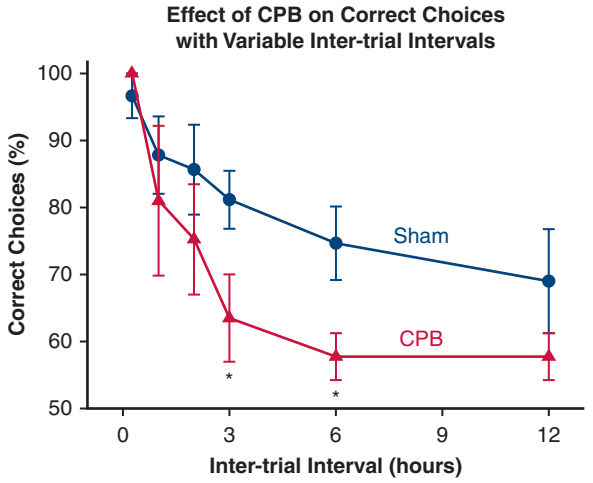

FIGURE 1. Impaired performance on the win-shift task by animals subjected to CPB. Animals in both groups acquired the task and performed similarly at short (0.25, 1, and 2 hours) inter-trial intervals. However, at longer intervals (3 and 6 hours), the CPB group performed significantly more poorly (*$P < .05$, 1-way analysis of variance). *CPB*, Cardiopulmonary bypass.

staining at 2, 4, and 7 days post-CPB did not reveal degenerating neurons (data not shown). In contrast, a significant increase in the number of activated microglia and a trend toward reduced numbers of immature neurons were observed at 6 months post-CPB (Figure 2). The timeline and fundamental outcomes of this study are summarized in Figure 3.

DISCUSSION

Our findings demonstrate that persistent cognitive dysfunction exists in an established rat model of CPB. It is notable that this CPB model has been shown not to produce neuronal loss,[3,4] a finding corroborated by our histologic outcomes. Consequently, it is reasonable to consider alternative, non-neurodegenerative mechanisms as potentially underlying the cognitive deficit. Two mechanisms that could possibly contribute to cognitive dysfunction are increased neuroinflammation and reduced adult neurogenesis. Our finding of a long-term increase in the number of activated macrophages/microglia after CPB

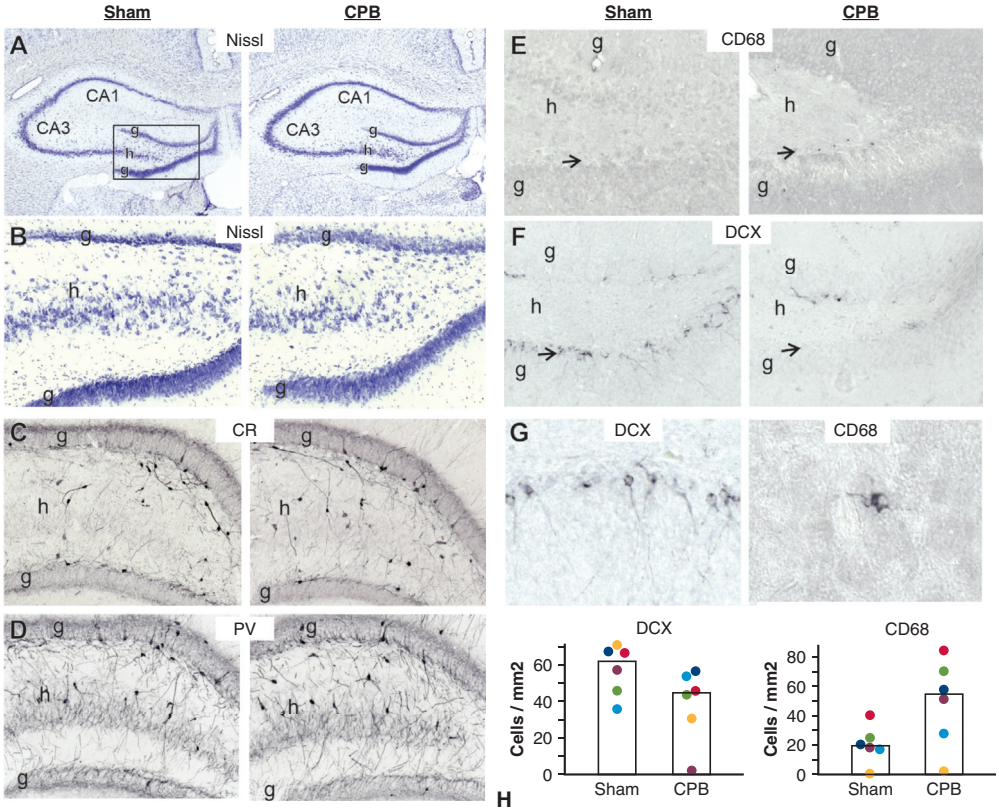

FIGURE 2. A-G, Tissue sections stained for cell bodies (*Nissl*), calretinin interneurons (*CR*), parvalbumin interneurons (PV), macrophages/activated microglia (*CD68*), and immature neurons (*DCX*) are shown. The rectangle in the left panel of A, which frames the dentate gyrus of the hippocampus, depicts the areas shown in B to F (g = granule cell layer; h = hilus). No apparent loss of primary neurons or interneurons was observed. In contrast, the population of activated microglia/macrophages appeared increased (E), and the population of immature neurons appeared decreased (F) in the CPB group (arrows indicate infragranular zone). G, Higher magnification images of DCX^+ and $CD68^+$ cells illustrate the typical morphology of immature neurons and activated microglia, respectively. H, Quantitative cell counting demonstrates a significant ($P = .04$, *t* test) increase of $CD-68^+$ cells and nonsignificant trend ($P = .09$, *t* test) for a decrease in DCX^+ cells in the CPB group. Graph bars are medians, and dots are individual animals. *CPB*, Cardiopulmonary bypass.

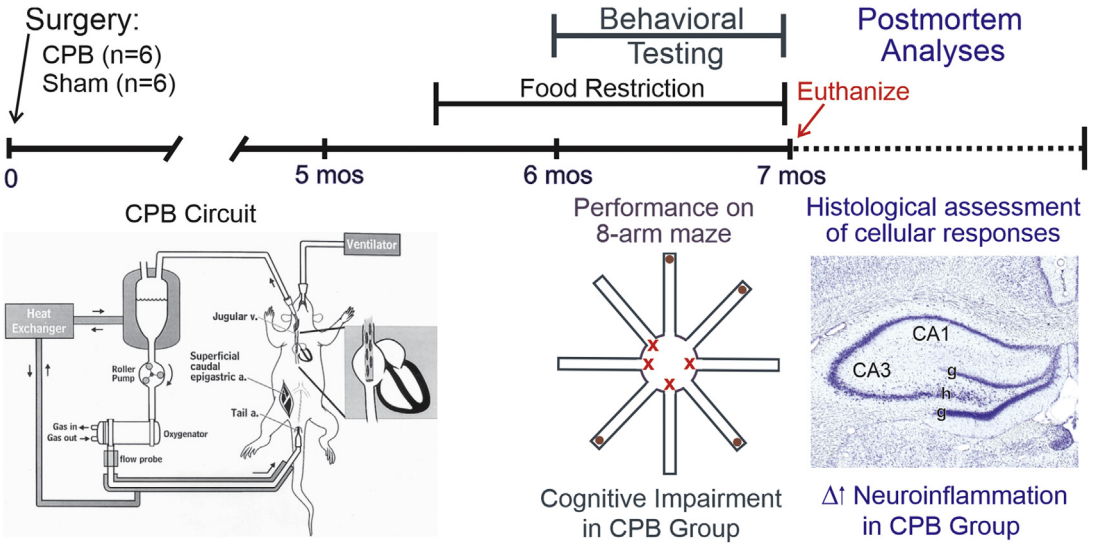

FIGURE 3. A time line of the study is shown. Surgery is performed on day 0 of the experiment, and the CPB circuit is illustrated (adapted from Grocott and colleagues[2]). At 5½ months post-CPB, food restriction is initiated. Starting at 6 months post-CPB, behavioral testing is performed using an 8-arm maze. Animals are euthanized at the end of behavioral testing via perfusion fixation, and cellular analyses on tissue sections are performed using histologic and immunohistochemical techniques. Cognitive impairment and increased neuroinflammation were key findings in the CPB group. *CPB*, Cardiopulmonary bypass.

is consistent with a sustained neuroinflammatory response. This response occurs in the infragranular zone of the dentate gyrus, an area that exhibits neurogenesis in adulthood. There was a trend toward fewer immature neurons after CPB. This is notable because alterations in adult neurogenesis can affect learning and memory. Clearly, other mechanisms contributing to cognitive compromise, such as disturbances in white matter, are possible and not ruled out by the current study. Nonetheless, our findings are parsimonious with the hypothesis that sustained neuroinflammation and perhaps reduced neurogenesis contribute to persistent cognitive dysfunction after CPB.

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APPENDIX E1. METHODOLOGY OF THE BYPASS PROCEDURE

All animal procedures were approved by the University of Virginia Animal Care and Use Committee. Male Sprague-Dawley rats (375-400 g) were subjected to a CPB procedure or a sham-CPB procedure. Six animals per group were used for behavioral testing, and this group size was based on previously published evidence using the same behavioral task.[5] Animals were fasted, but allowed free access to water for 12 to 14 hours before surgery. Anesthesia was induced with 3% halothane, the trachea was intubated, and the lungs were mechanically ventilated. Anesthesia was maintained with 1.0% to 1.5% halothane, with surgery performed using aseptic techniques. The CPB circuit is identical to that described by Grocott and colleagues.[2] Briefly, the tail artery was cannulated to serve as the inflow for the CPB circuit. A dual-stage venous cannula was inserted in the internal jugular vein and advanced until the tip of the cannula was located near the junction of the inferior vena cava and right atrium. With the tip of the venous cannula in this position, drainage of the right superior vena cava, left superior vena cava (which is found routinely in rats), and inferior vena cava is optimized. The rat possesses both left and right superior vena cavae, allowing the right to be ligated without adversely affecting cerebral venous outflow. The CPB circuit consists of a venous reservoir, a peristaltic pump, a membrane oxygenator, and the arterial inflow, and then proceeds back into the animal via the arterial inflow cannula. An in-line flow probe was used to measure CPB flow continuously. The flow was 160 to 180 mL/kg/min, which is similar to the normal cardiac output in rats. Arterial line flow temperature was maintained at 37.5°C using a circulating water bath. The CPB circuit was primed with approximately 40 mL of whole blood from heparinized donor rats, which were exsanguinated under deep anesthesia. Venous oxygen saturation from the venous return line was measured continuously using an Oximetrix monitor and Opticath catheter (Abbott Labs, Chicago, Ill). Arterial blood gas analyses and hemoglobin levels were also monitored at 20-minute intervals. After the surgical preparation, local anesthetic was infused into the wounds, which were then closed around the catheters. Adequate depth of anesthesia was ensured, as detailed by Mackensen and colleagues.[3] Animals were randomly assigned to the CPB or sham-operated group. CPB was maintained for 60 minutes. Atelectasis during CPB was prevented by not ventilating the lungs, but by providing 5 mm Hg of continuous positive airway pressure and keeping the fraction of inspired oxygen at 0.21. After CPB, animals were weaned from CPB with inotropes or vasopressors. After decannulation, rats remained anesthetized, intubated, and ventilated for 2 hours, after which any residual neuromuscular blockade is reversed with neostigmine (50 μg/kg, intravenously) and glycopyrrolate (20 μg/kg, intravenously). The trachea was extubated when animals resumed spontaneous ventilation. Recovery took place in an oxygen-enriched environment for 24 hours with free access to water and food. The sham group had all CPB catheters left in place for the same 60-minute period as the CPB group, but were not hooked up to the external CPB circuit. Decannulation and extubation were performed in the same manner as the CPB group. All animals were carefully monitored for the next week.

Animals were allowed free access to food and water for the next 5½ months, after which food access was restricted to reduce an animal's weight to 90% of its ad libitum feeding weight. Food restriction was continued through the end of behavioral testing to maintain adequate motivation to perform the win-shift task. Behavioral testing began 6 months post-CPB. It is important to note that nutrition is one of many variables that could affect functional outcomes after CPB.[6] However, animals in the CPB and sham groups were treated identically, in terms of feeding, during all stages of the study. A key future benefit of the current study is that it provides specific behavioral and cellular outcomes for studying the effects modifying systemic variables (eg, presurgical or postsurgical nutrition) on post-CPB outcomes.