

## Allogeneic Head and Body Reconstruction: Mouse Model

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Body reconstruction; Composite tissues  
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### SUMMARY

**Aims:** There is still no effective way to save a surviving healthy mind when there is critical organ failure in the body. The next frontier in CTA is allo-head and body reconstruction (AHBR), and just as animal models were key in the development of CTA, they will be crucial in establishing the procedures of AHBR for clinical translation. **Methods and results:** Our approach, pioneered in mice, involves retaining the donor brain stem and transplanting the recipient head. Our preliminary data in mice support that this allows for retention of breathing and circulatory function. Critical aspects of the current protocol include avoiding cerebral ischemia through cross-circulation (donor to recipient) and retaining the donor brain stem. Successful clinical translation of AHBR will become a milestone of medical history and potentially could save millions of people. **Conclusions:** This experimental study has confirmed a method to avoid cerebral ischemia during the surgery and solved an important part of the problem of how to accomplish long-term survival after transplantation and preservation of the donor brain stem.

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### Introduction

Composite tissue allo-transplantation (CTA) involves the grafting of limbs or other complex tissues from an unrelated donor and recipient. In the 1990s, animal studies helped to pave the way for the first successful human hand transplantation in United States, which was performed at the University of Louisville and Christine M. Kleinert Institute for Hand and Microsurgery in 1999 [1–5]. This patient recovered fully, and continues to work and lead a normal social life [6–8]. Studies in small animals and a porcine model allowed for optimization of the immunosuppressive regimen, as well as a system for characterizing the degree of immune rejection of transplanted tissues. Facial tissue transplantation has also become a clinical reality [9–13], and worldwide there have been more than 100 completed cases of the CTA operation [14–17]. However, there is no effective way in which to save a survival healthy mind when there is critical organ failure in the body, such as complete cervical spinal cord injury with paraplegia, tumors metastatic disease, hereditary body muscle atrophy, and others.

If a part of the body as important and complex as a hand can be transplanted successfully, it is natural to ask whether the most important part of the body, the head, could also be transplanted. This is the next frontier in composite tissue transplantation. A number of animal models of head transplantation have been

developed over the last century, beginning with that of dogs in the former Soviet Union in the 1950s by Dr. Demichow [18–20]. More clinically applicable studies followed in the 1970s, when Dr. Robert White and colleagues successfully transplanted the head of a rhesus monkey between the 3rd and 4th cervical vertebrae. While this represented important progress in understanding anatomical and physiological aspects of head transplantation [21–23], these models did not evaluate any measures to prevent immune rejection and did not have an effective strategy for central nervous system recovery. More recently, the Italian neurosurgeon Dr. Sergio Canavero has proposed a surgical strategy referred to as the HEAVEN procedure, which preserves brain function through hypothermia during the transplantation procedure, which is performed at cervical level C5/6. He presents a possible way to connect the recipient and donor spinal cords using inorganic polymers to allow the cell membranes of the donor and recipient axons to fuse [24].

Another new strategy known as allogeneic head and body reconstruction (AHBR) has also recently been proposed [25,26]. In this technique, which is based on the practice of CTA, the donor body is the transplanted composite tissue. In this procedure, brain ischemia is avoided not by deep hypothermia, but by maintaining adequate blood pressure and circulation between the recipient and the donor body, through anastomosis of the

donor and recipient carotid arteries and jugular veins during surgery. Furthermore, this procedure preserves the donor body's brain stem, which allows for spontaneous postoperative respiratory and cardiovascular function. This research describes how the AHBR model is being implemented and tested in mice, in preparation for the development of a primate model that will help to evaluate the clinical applicability of AHBR.

## Methods

### Animals

The experiments were approved by The Animal Care and Use Committee of The Second Affiliated Hospital of Harbin Medical University. Forty Kunming mice and forty C57 wild type mice, all male, age 10–15 weeks, weight  $30 \text{ g} \pm 5$ , were obtained from the animal center of Harbin Medical University.

### Experimental Drugs and Equipment

The following drugs were employed as follows: 3% sodium pentobarbital (90 mg/kg), heparin sodium injection (1.0 mL/100 mg), and norepinephrine (5 mg/kg). During surgery, respiration was maintained using the Minivent Mouse Ventilator Type 845 (Hugo Sachs Elektronik Harvard Apparatus GmbH D-79232 March, Germany). An Operating Microscope (SXP-1C) from Shanghai Medical Optical Instruments CO, Ltd., Shanghai, China, was used along with a sixteen-channel physiological recorder PHY-001 (BIOPAC MP-150, Goleta, CA, USA), the intelligent noninvasive blood pressure measurement analysis system (BP-98A, Softron, Tokyo, Japan), and an electrosurgical generator (HV0300A), Beijing Heng Wei Technology Development CO, Ltd., Beijing, China. Micro- and neurosurgical instruments and surgical supplies were purchased from the Shanghai Instrument Company (Shanghai, China).

### Monitoring of Vital Signs

Perioperative and postoperative EEG and ECG were monitored. Blood pressure and body temperature were monitored to regulate the body's metabolic condition. The ventilation was set to the rate of  $100 \pm 5$  respirations per minute with a tidal volume of 2.2 mL.

After surgery, special attention was given to breathing regularity and frequency as well as ECG indicators because the donor brain stem may have limited physiological function. Nerve reflexes and basic sense of movement and muscle tension were regularly checked and recorded.

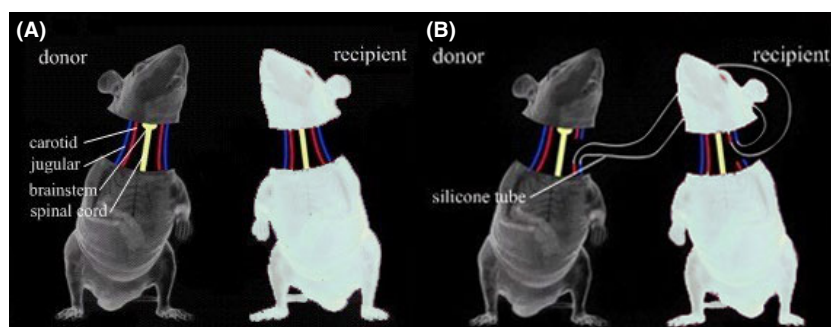
### Preparation of the Donor Body

A ventral circular incision was made at cervical level C3–C4. The skin and subcutaneous tissue were incised, and muscle, nerves, blood vessels, trachea, and esophagus were dissociated. Tracheal intubation was performed. Next, the distal ends of the carotid and jugular vessels were ligated and the proximal ends on one side of the body were catheterized using a 0.30 mm (I.D.)  $\times$  0.64 mm (O.D.) silicone tube to create an anastomosis with the ipsilateral carotid and jugular vessels from the head of the recipient (Figure 1), and flushed with heparin sodium injection (1.0 mL: 100 mg) mixed with sodium chloride injection. The proximal ends of the carotid and jugular vessels on the other side of the body were ligated.

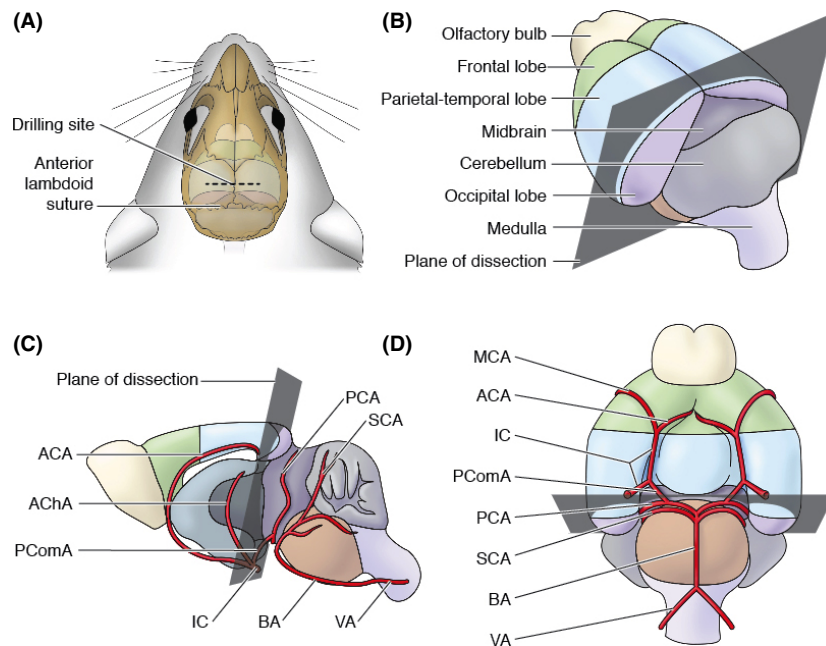
Next, a circular incision was made on dorsal aspect of the head at the level of the midbrain. The scalp and galea aponeurotica were incised to expose the skull. To preserve the integrity of the midbrain and avoid the circle of Willis, a location was selected 3 mm distal to the anterior lambdoid suture, perpendicular to the sagittal suture. After drilling using a dental drill, a sharp cut was made at the level of the midbrain (Figure 2) [27]. Importantly, placing the incision at this level preserves the donor brain stem, permitting postoperative spontaneous, independent breathing and circulation. All surgical procedures were completed under the microscope and bleeding was stopped by bipolar coagulation.

### Preparation of the Recipient Head

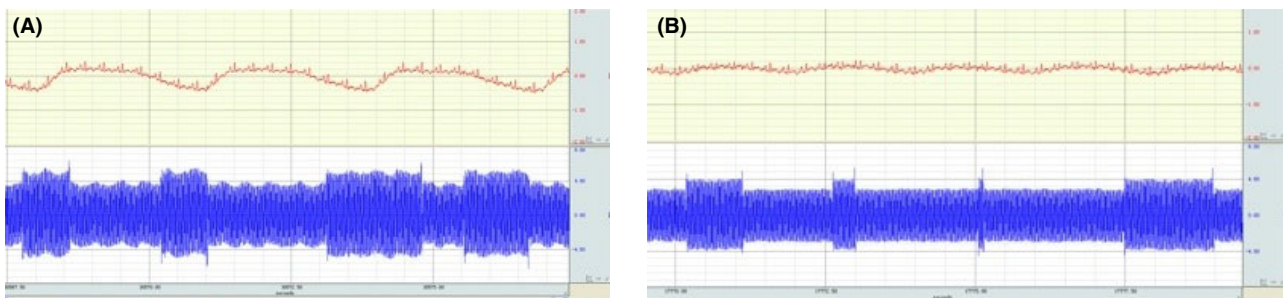
Ventral and dorsal incisions weremade at the cervical level C3–C4. The skin and subcutaneous tissue were incised, and muscle, nerves, blood vessels, trachea, and esophagus were dissociated. The vertebrae were exposed, and the arteries and veins around the spinal cord were ligated at level of C3–C4. The spinal cord was cut sharply. The proximal ends of the carotid and jugular vessels were ligated. The distal carotid artery and jugular vein were catheterized as before to connect with the ipsilateral carotid and jugular



**Figure 1** Schematic representation of the AHBR mouse model. (A) Two mice with different coat colors were selected. All tissues were separated including carotid artery, jugular vein, spinal cord, and so on. (B) To ensure transplanted brain tissue undergoes continuous blood circulation to avoid cerebral ischemia and hypoxia, crosscirculation is established by the silicone tubes.



**Figure 2** Location of the cutting plane in donor mice. (A) Drill 3 mm distal to the anterior lambdoid suture, (B) superolateral aspect of the brain showing the cutting plane, (C) sagittal section, and (D) inferior aspect of the brain showing the cutting plane and vasculature. The cutting plane passes from the caudal edge of the parietal–temporal lobes, along the rostral aspect of the midbrain, and through the PcomA. This preserves blood flow to the regions of the donor’s brain that are retained. ACA, anterior cerebral artery; AChA, anterior choroidal artery; PcomA, posterior communicating artery; IC, internal carotid; MCA, middle cerebral artery; PCA, posterior cerebral artery; SCA, superior cerebellar artery; BA, basilar artery; and VA, vertebral artery.



**Figure 3** AHBR mouse model (electroencephalographic and electrocardiography recording). (A) The record of ECG and EEG preoperation and (B) postoperation.

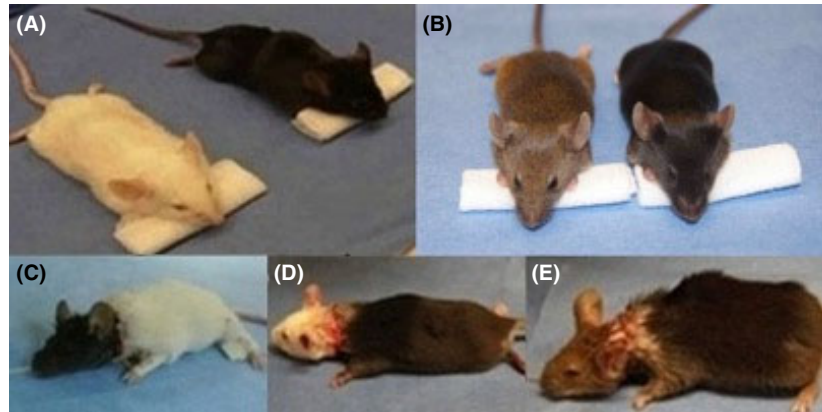
from the donor body. Likewise, they were flushed with heparin sodium injection (1.0 mL: 100 mg) mixed with sodium chloride injection. The distal ends of the carotid and jugular on the other side were ligated.

With the completion of osseous separation, the Cephalon was associated with the isolated body only through bilateral carotid–jugular vascular loops, which provided cerebral blood flow. Finally, surgical stitching was used to appose donor and recipient tissues including spinal cord membrane, bilateral carotid arteries, bilateral jugular veins, trachea, esophagus, muscles, subcutaneous tissue, and skin.

## Results

Forty Kunming mice and forty C57 wild type underwent the AHBR procedure. After transplantation, 18 mice survived

for 3 h after the ventilator was disconnected. As anticipated, they were capable of breathing spontaneously at a normal rate, because the donor brain stem remained intact. The rate of respiration was  $140 \pm 15$ /min. During these 3 h, the mice awakened and displayed normal cranial nerve function and characteristic responsiveness (blinking, whiskers moving, etc.). After the mice awoke, electroencephalogram (EEG) recordings were made directly from the cortex of the transplanted Cephalons (Figure 3). Because blood supply was maintained at an adequate level during surgery by anastomosing the donor and recipient carotid and jugular vessels, the intra- and post-operative EEG and ECG show electrophysiological activity. The systolic blood pressure was maintained above 50 mm/Hg (measured by tail artery). Although ECG monitoring shows an unstable curve postoperatively, there was neither significant tachycardia nor bradycardia, and no lethal arrhythmia.



**Figure 4** Three pairs of mice before and after surgery. (A and B) Black, white, and brown mice; (C–E) mice after transplantation (black head with white body, white head with black body, and brown head with black body).

The heart rate was  $300 \pm 20$ /min. Interestingly, the mice did not show postoperative decerebrate rigidity (Figure 4).

## Discussion

The brain stem is home to many reflex centers that are required for basic life functions, including cardiovascular and respiratory. As seen in Figure 1, after transplantation, the donor body continues to have a regular heart rate, which supports the hypothesis that retaining the donor's brain stem allows the body to regulate its own cardiovascular function. In animal models where the brain stem is not preserved, that is, in models using C3/4-level transection, independent breathing and circulation are lost, and survival must depend on life-support machines. Our short-term results so far show that retaining the donor brain stem allows the animal to recover both independent breathing and cardiovascular function.

Furthermore, the recipient's brain has continued electrical activity in the postsurgical EEG, which suggests that the donor's heart was able to provide sufficient blood flow to the recipient's brain via the cannulae placed during surgery. White's research described the need for maintaining blood pressure of at least 40 mmHg to support the metabolic needs of brain tissue. In the animal model described herein, the postoperative blood pressure is up to 50 mmHg. This is important because the brain is the most metabolically active organ in the body, making it very intolerant of ischemia. The preoperative temperature of mice is  $31 \pm 2^\circ\text{C}$ , which is sufficient to

meet the body's metabolic needs. As such, this body temperature was maintained during surgery.

Future studies will use electromyography (EMG) to test the action potential of muscles and extend the survival of the mice for a longer time [28]. This will allow signs of immune rejection to be monitored while using immunotherapy. Once the mouse model has been validated, we will build upon it to establish a primate model; these models will be crucial for making translation of this technique to the clinic possible [29,30].

In summary, our short-term results have been promising. We have confirmed a method to avoid cerebral ischemia during the surgery and solved an important part of the problem of how to accomplish long-term survival after transplantation and preservation of the donor brain stem. If AHBR can be successfully translated into clinical practice, it has the potential to save countless critically ill or injured patients' lives, which will be a monumental achievement in the history of medicine.

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## Conflict of Interest

The authors declare no conflict of interest.

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