Isolation of the Monkey Brain: In vitro Preparation and Maintenance

Abstract. *Sustained viability of the primate brain, as a totally isolated organ preparation, was achieved by utilizing an extracorporeal (compatible donor) circulation. Five rhesus monkey brains, completely isolated neurogenically and vascularly, were perfused in vitro for 30 to 180 minutes. Retention of biological activity was evidenced by: (i) persistent electrocortical activity, and (ii) significant mean A-V_{O_2} (5.8 volumes percent) and V-A_{CO_2} (5.0 volumes percent) differences across the isolated brain.*

The brain, because of the complex formation of its circulation and the rapidity of its biological decay, has not been successfully prepared or maintained as a completely isolated organ. While the feasibility of protracted survival of the severed animal head and, indirectly, of the brain, as evidenced by the retention of primitive reflexes, has been demonstrated in the dog (*1*) and cat (*2*) during vascular perfusion, in vitro studies of pure cerebral function and metabolism uninfluenced by contiguous structures have previously been limited to cerebral tissue slices (*3*) or homogenates (*4*).

In order to assure a totally isolated brain preparation the following two criteria would have to be met: (i) the arterial circulation be directed exclusively to the brain, and (ii) the venous circulation arise solely from the brain. In order to achieve these two basic requirements, it seemed to us that only the surgical elimination of all active metabolic tissue surrounding brain and the exclusive preservation of the cerebral circulation would accomplish these ends. In the monkey, because of the relative simplicity of the formation of its intracranial circulation from the extracranial vasculature, these requirements are most easily met for an isolated brain preparation. In addition, the relatively large size of the internal carotid arteries in this species suggested that bilateral internal carotid perfusion would suffice to maintain cerebral circulation if the vertebral circulation was purposely eliminated (*5*). Since it is mandatory to attest to the viability of the isolated brain, the recording of electrical cortical potentials appealed to us as the simplest and most direct method of monitoring biological activity in this preparation.

Ten acclimatized rhesus monkeys in two weight ranges, 6 to 8 lb (2.7 to 3.6 kg) for the isolated brain preparations and 15 to 25 lb (6.8 to 11.3 kg) for the perfusion donors, were used. The isolated-brain monkey and the donor animal were crossmatched prior to surgery to assure blood compatibility. The isolated-brain monkey was anesthetized with sodium pentobarbital and a tracheostomy was carried out with the introduction of a cuffed, wire-spiral, endotracheal tube. The monkey was immobilized with Flaxedil (gallamine triethiodide) and ventilated with 100-percent oxygen by a Harvard respirator. A femoral artery was cannulated for arterial pressure measurements. The large donor monkey was similarly anesthetized and ventilated, except that oral tracheal intubation was performed. The brachial artery was utilized in this animal for intra-arterial pressure monitoring and the femoral artery and vein cannulated for perfusion. All pressure recordings were made on an oscillographic recorder utilizing strain gauges.

Operative preparation of the isolated-brain monkey consisted of removal of all anatomic structures surrounding the brain save for a small basal plate of bone and the central portion of the skull, in which were mounted three pairs of silver disk electrodes resting on the surface of the dura and connected to a 4-channel Grass Polygraph with electroencephalographic preamplifiers (Fig. 1). Anatomically, only the internal carotid arteries and jugular veins were preserved, since all extracranial vasculature was simultaneously eliminated with the surgical removal of the jaws, orbits, and nasal and oral structures. After cervical laminectomy the spinal cord was ligated and, together with the vertebral column, divided between C_1 and C_2. The vertebral arteries were occluded just prior to their formation of the basilar artery. This resulted in a closed system of perfusion through the Circle of Willis. Extracorporeal circulation after heparinization was accomplished from the donor's femoral artery to two small metal "T" cannulae placed in the carotid arteries. Venous return from the brain by way of the jugular veins and basal sinuses was collected in a reservoir and returned to the donor's femoral vein through a small, previously calibrated, occlusive pump (Fig. 1).

During the surgical preparation of the isolated-brain monkey the mean arterial pressure of the monkey was maintained between 120 and 140 mm-Hg by the addition of small increments of dextran or compatible donor blood. With the institution of perfusion there was a fall in the systemic arterial pressure of the donor averaging 20 to 30 mm-Hg; however, when venous return

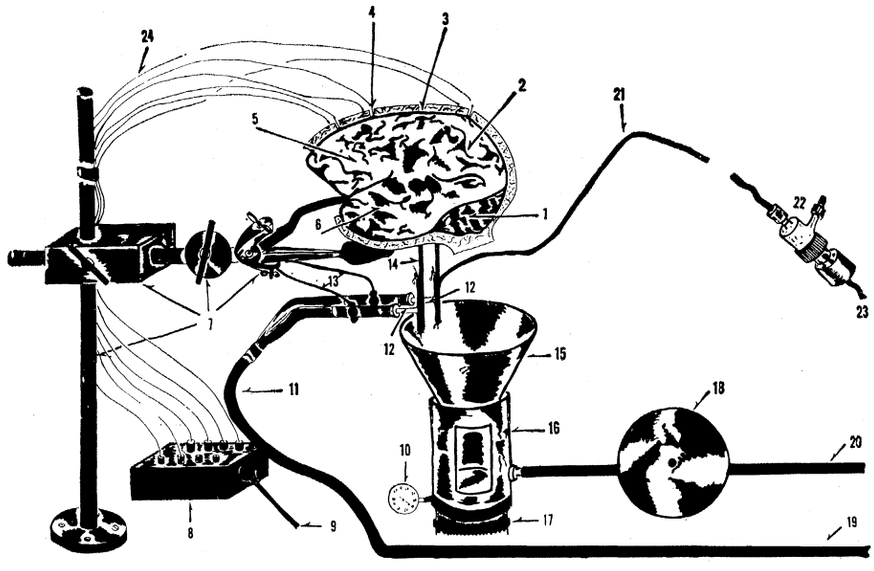

Fig. 1. The isolated monkey brain during donor perfusion. *1*, cerebellum; *2*, occipital lobe; *3*, strip of skull containing electrodes; *4*, cortical electrodes; *5*, frontal lobe; *6*, temporal lobe; *7*, orbital-oral fixation unit; *8*, EEG plug-in-box; *9*, electrode cable to EEG preamplifiers; *10*, venous reservoir thermistor; *11*, arterial line; *12*, carotid arteries containing metal "T" cannulae; *13*, wire "stirrups" to support arterial cannulae; *14*, internal carotid arteries; *15*, collecting funnel for venous reservoir; *16*, venous reservoir; *17*, heating unit for reservoir; *18*, occlusive venous pump; *19*, arterial line from femoral artery of donor; *20*, venous line to femoral vein of donor; *21*, arterial pressure cannula; *22*, pressure transducer; *23*, cable connection to oscillographic recorder; and *24*, EEG leads from isolated brain.

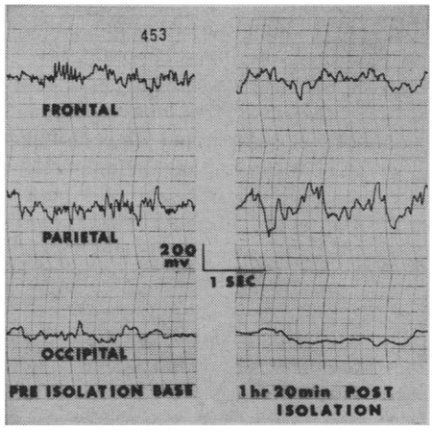

Fig. 2. A sampling of electrocortical activity from the frontal, parietal, and occipital lobes of the first monkey brain successfully maintained as a totally isolated organ is reproduced. The first recording was obtained prior to neurogenic and vascular isolation; the second was made 1 hour and 20 minutes after total isolation and the institution of extracorporeal donor perfusion. The slowing and reduction in electrical activity (particularly over the occipital regions) in the post-isolation tracing was considered to be due, in part, to the brain's low temperature (31°C).

was established between the reservoir and the donor monkey the arterial pressure rose to preperfusion levels of 160/120 to 190/140 mm-Hg. Internal carotid pressure measurements via the "T" cannula (Fig. 1) of the isolated brain were 20 to 30 mm-Hg lower than the donor's systemic arterial pressure during perfusion. Perfusion rates in the totally isolated monkey brain (average weight 83.5 g) ranged from 20 to 60 ml/min as measured by venous return.

Serial electrocortical tracings revealed persistence of electrical activity for periods ranging from 30 to 180 minutes during isolated brain perfusion (Fig. 2). With total cessation of perfusion the electrocortical tracings became flat within 30 seconds. Direct inspection of the cortex of the brain during perfusion demonstrated normal appearing cortex, arteries, and veins. This last observation was supported by the mean $A\text{-}V_{O_2}$ and $V\text{-}A_{CO_2}$ differences across the isolated brain, which averaged, respectively, 5.8 vol. percent and 5.0 vol. percent after 2 hours of perfusion. Intracerebral temperature recordings made in the frontal and parietal lobes of the isolated brain indicated a persistent hypothermia (30° to 35°C) with an excellent agreement (within 0.5°C) between the anterior, posterior, superficial, and deep areas of cerebrum.

The major problem in the preparation of the isolated monkey brain has been the design of an operation which would completely isolate the brain and yet permit the total organism to be maintained in a satisfactory physiological state until circulatory bypass was begun. This was achieved by meticulous surgical technique and careful maintenance of circulating blood volume. In each of the five isolated-brain perfusions, the experiment was terminated because of marked reductions in the hematocrit (mainly due to repeated arterial blood sampling) of the total circulating system. The desire here was to secure the survival of the large, costly donor monkey for future experiments. While there is reduction in the electrical activity recorded from the isolated brain (Fig. 2), particularly over the occipital cortex, some of this reduction was felt to be due to the existing cerebral hypothermia and the post-perfusion finding that the cortex, particularly the occipital cortex, fell away from the skull. Further evidence of active metabolism in the isolated monkey brain was demonstrated by the significant $A\text{-}V_{O_2}$ and $V\text{-}A_{CO_2}$ differences, which compare favorably with those reported for the monkey brain *in situ* (*6*).

Previous attempts to vascularly "isolate" the mammalian brain *in situ* or as an isolated head preparation have principally relied upon simple ligation of the extracranial vessels in order to eliminate arterial and venous contamination. In the ingenious "isolated" cat brain of Chute and Smyth (*2*) extracranial tissues were responsible for 14 percent of the measured metabolism of this preparation. Likewise, the metabolic data obtained from the *in situ* "isolated" cat brain of Geiger (*7*) has been criticized because of incomplete vascular isolation and the use of heterologous perfusion media (*8*).

These experiments demonstrate for the first time the feasibility of protracted survival of the subhuman primate brain as a totally isolated organ utilizing extracorporeal donor perfusion (*9*).

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9. Supported by research grant No. 5455M3817 from the National Institute of Neurological Diseases and Blindness.

19 July 1963

Circulating Interferon in Mice after Intravenous Injection of Virus

Abstract. *Circulating interferon was detectable in mouse serum within 1 hour after the intravenous injection of various types of virus and it reached maximum levels in about 4 hours. Rapidly produced interferon may play a role in the pathogenesis of viral infection and in viral interference.*

Interferon is thought to be an important antiviral factor in the recovery of animals from viral infection (*1*). Although the production and antiviral action of interferon is generally localized at the site of viral infection (*2*), indirect evidence suggests that small amounts of interferon may pass from infected tissues to distal sites (*3*). However, circulating interferon was not consistently detected in these studies. To test the possibility that circulating interferon might be produced as a result of viremia occurring during infection, mice were injected intravenously with virus and subsequently their serums were assayed for interferon.

Interferon was assayed by the plaque-reduction method (*4*) in which primary cultures from mouse embryos were used; the cultures were treated for 24 hours with 3 ml of two- to four-fold serial dilutions of interferon and then challenged with 20 to 60 plaque-forming units (pfu) of vesicular stomatitis virus (VSV). Interferon titers, expressed as units per 3 ml, were determined as the dilution of the original material required to reduce the plaque count to 50 percent of that of the controls. Newcastle disease virus and Sendai virus were grown in the allantoic sac of chick embryos aged 9 to 11 days; vaccinia virus was propagated in