Instrumental Responses in Rats After Hypothermic Cardiopulmonary By-Pass.* (32408)

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The evaluation of damage caused in man (1,5) and animal(6,7) by heart-lung by-pass has been somewhat difficult, because one of the standard laboratory animals, the rat, has not lent itself for testing because of its small size. Recently we developed in our laboratory a miniaturized heart-lung machine, which has made it possible to support 70% of cardiac output and 100% oxygenation in hypothermic rats. The purpose of this study is to use hypothermic rats, in which, because of their low metabolism, sufficient oxygenation is assured for testing highly integrative cerebral activity, the instrumental response (rate of intracranial self-stimulation) before and after by-pass.

Materials and methods. Bipolar stainless steel electrodes, insulated to within 0.25 mm of the tip, were chronically implanted in 30 rats (Sprague-Dawley) under Nembutal anesthesia in the supramammillary area of the hypothalamus ("pleasure center") by means of a stereotaxic instrument(8). The electrodes were held in place by self-curing acrylic compound. Three small screws served to anchor the acrylic material to the skull and to immobilize the electrodes. During the same operation a small 40 ga copper-constantan thermocouple was placed under the skin on the back or in the brain. Four days later each animal was placed in a self-stimulating chamber (Fisher) and trained to self-stimulate intracranially (Fig. 1). Exploring the box, the animal pressed the lever accidentally at an average of 0.8 time per minute, thus stimulating its brain. During the next 10-15 minutes the number of lever pressings increased steadily reaching a maximum at the end of this period. After the first exposure of one hour each rat was placed in the self-stimulating box 4 more times, one hour every other day. Though the rate of performance varied from animal to animal, the rate of performance of each rat was almost the same from one day to the other, the mean value of the whole group being the same, while the standard deviation of the mean was increased for 2% only. The current of each stimulus was 50-85 μA and lasted 0.4 second.

The animals were divided into 3 groups, two control groups and one experimental group.

Group 1. Control hypothermic rats with respiratory arrest. After 4 training sessions for self-stimulation 8 rats were cooled(9) to a body temperature of 13-14°C. At this temperature the respiration of the animals ceased either immediately or within 5-10 minutes. One hour later 4 of the animals were rewarmed to 37°C, whereas the other 4 animals were rewarmed after a 4-hour hypothermic period.

Group 2. Control hypothermic rats with spontaneous breathing. After 4 training sessions, 8 rats were cooled as were the above group, though only to 16°C. Four of the animals were kept at this temperature for one hour whereas the other 4 were kept for 4 hours. All animals continued to breathe during the hypothermic period. The rats were rewarmed to normothermia at room temperature.

Group 3. Hypothermic rats with respiratory arrest on 1- to 4-hour cardiopulmonary by pass. After 4 training sessions 14 animals were cooled to 16°C. At this moment each animal was placed on a refrigerated operating table in order to keep the body temperature low while the aorta and right ventricle of the animal were cannulated(10). The aortic cannula consisted of a PE 90 tubing (i.d. 0.86 mm) while the right ventricular cannula was made of PE 200 tubing (i.d. 1.40 mm). After cannulation the animals

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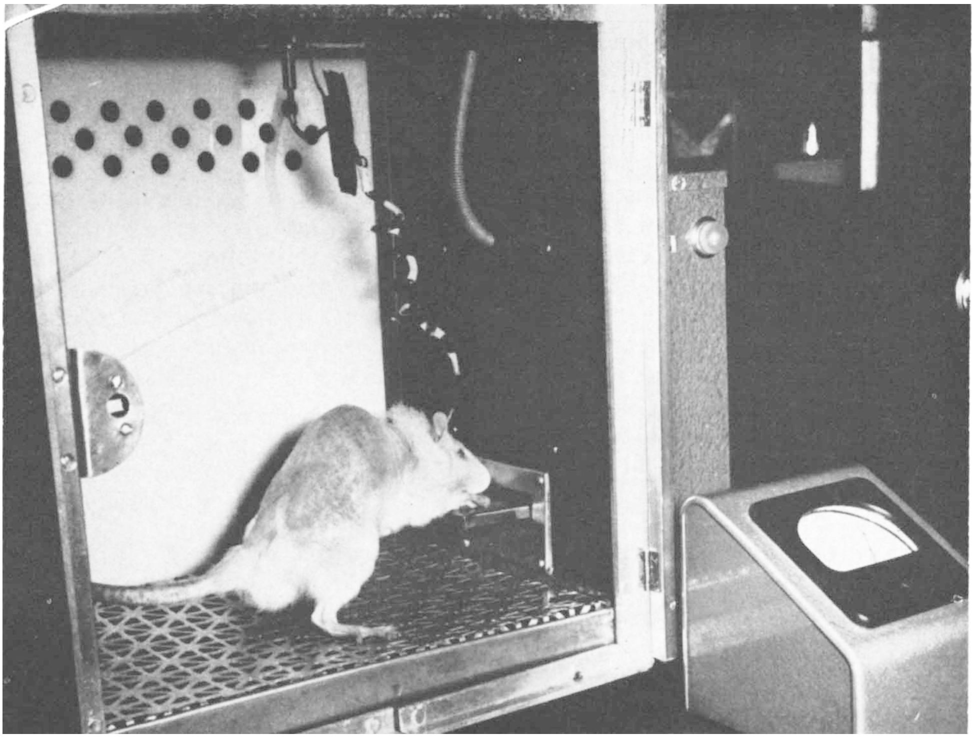


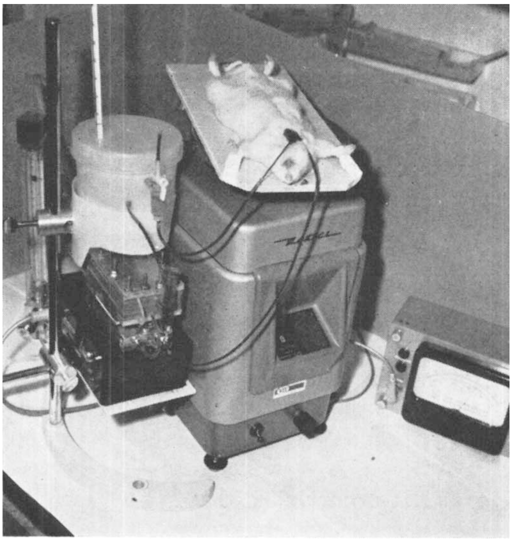
FIG. 1. Apparatus for intracranial self-stimulation in rats.

were cooled further, to a body temperature of 14°C, which resulted in respiratory arrest. At this moment each rat was connected to a heart-lung machine and partial cardiopulmonary by-pass was initiated.

The heart-lung machine was developed in our laboratory. It consisted of a roller pump, a membrane type oxygenator, a filter-bubble trap unit, a venous reservoir and connecting tygon tubings (Fig. 2).

A micro bilateral roller pump (Model RD 045, Holter Co.) was used for the extracorporeal shunt, while the blood from the right ventricle was drained by gravity into the oxygenator (Fig. 3), where the blood was arterialized in 8 parallel blood chambers. The oxygen entered blood through silastic membranes from parallel oxygen chambers supported by silicon rubber mats. The total surface area of all membranes was 650 cm^2. The total priming volume of the oxygenator was only 8.0 ml (168.3 ml m^2 of membrane). Pulsatile flow of 350 ml/min of pure oxygen was used. The heat exchanger was made of spiral stainless steel. The filter bubble trap was placed in the arterial line of the by-pass. The venous reservoir was calibrated and used for measurements of the volume blood flow and for compensation of the perfusion in the initial stages of the experiment. The connecting tubings were made of tygon. The total priming volume of the whole system was 12.1 ml (oxygenator 8.0, venous reservoir 1.0, heat exchanger 0.8, filter-bubble trap 0.8, tygon tubes 1.5 ml). The priming blood was obtained from a donor rat and was tested for compatability before being used. During perfusion the total priming volume was increased by 2 ml as a result of deformation of the membranes in the oxygenator. The blood volume was kept constant by continuous weighing of the perfused animal and adjustments of pump output for "balanced perfusion"(5,11). The body temperature of the animal was kept constant by immersion in cold water and by cooling of the blood in the heat-exchanger.

The volume of the blood flow was 28 ml/kg/min. This is about 70% of the total cardiac output at a body temperature of



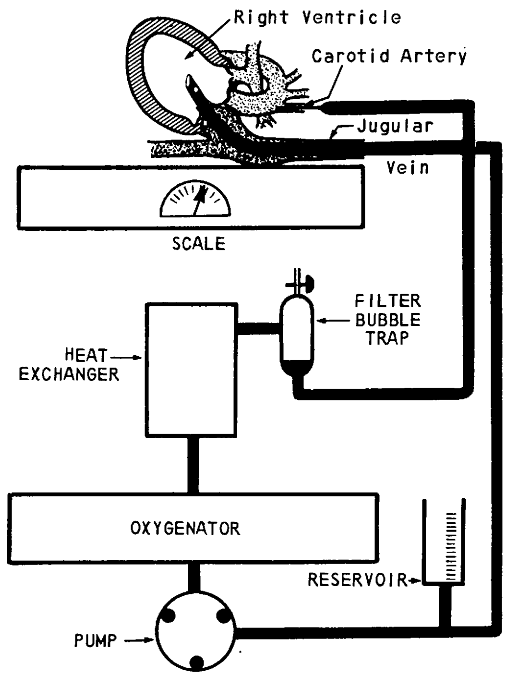


FIG. 2. Left, schema of heart-lung machine. Right Apparatus for extracoropreal circulation in rats.

14°C. The delivery of oxygen was 1.7 ml/kg/min, amounting to 100% of the total oxygen consumption of the animals at this temperature. The cardiopulmonary by-pass lasted one hour for 8 rats and 4 hours for 6 animals.

After the by-pass, all animals were rewarmed to normothermia. On the next day they were placed in the self-stimulating box to measure the rate of their intracranial self-stimulation.

Results. Group 1. Control hypothermic rats with respiratory arrest. All 8 rats in this group died during rewarming.

Group 2. Control hypothermic rats with spontaneous breathing. Eight animals trained to self-stimulate were cooled and kept 1 to 4 hours at 16°C. At this body temperature all animals had slow but normal spontaneous respiration. After rewarming all animals survived. One day later the animals were placed in self-stimulating box. Their rate of self-stimulation was similar to that before cooling. The mean value of the rate of stimulation was for only one lever pressing smaller than before cooling, while for standard deviation of the mean was slightly increased. These apply to the values of the rate of intracranial self-stimulation obtained at the next 3 sessions.

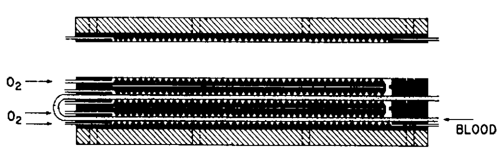


FIG. 3. Schema of the artificial lung for rats.

Group 3. Hypothermic rats with respiratory arrest on 1- to 4-hour cardiopulmonary by-pass. After being cooled to 14°C, which induced respiratory arrest, and then having been exposed 1 hour (8 animals) or 4 hours (6 animals) to hypothermic cardiopulmonary by-pass the animals were rewarmed to a body temperature of 37°C. All animals survived. On the next day the rats were placed in the self-stimulating box. All animals self-stimulated and the rate of self-stimulation was 46 ± 4 per min, similar to the one observed before cooling, *i.e.,* 47 ± 3. The animals were tested 3 more times (every other day). Their rate of the intracranial self-stimulation was the same as before by-pass.

Discussion. Temporary or permanent neurological changes after cardiopulmonary by-pass have been reported on several occasions (12,13). It is not clear whether these changes were caused by insufficient perfusion during normothermic cardiopulmonary by-pass, which has to support a high metabolic level of normothermic tissues, or by damage from extra-corporeal circulation techniques. To find out which one, if any, of the two possibilities causes neurological alterations after the normothermic by-pass, hypothermic by-pass was used in hypothermic rats. In this way the need for high volume blood flow was eliminated and the possibility of an adequate oxygen supply to the tissues was improved. For evaluating the presence or absence of neurological changes white rats were chosen because they were quite suitable for psychophysiological tests. The rats had permanently implanted electrodes in the supramammillary body of the hypothalamus. This permitted accurate measurement of a suitable instrumental response (performance rate of intracranial self-stimulation) for comparison of the findings in animals before and after hypothermic cardiopulmonary by-pass. Our experiments showed that the rate of instrumental performance in rats with electrodes implanted in the "pleasure center" was the same after hypothermic by-pass as it was before by-pass, indicating that no neurological disorders developed when adequate hypothermic by-pass was employed.

It can be concluded that the rats cooled to a body temperature of 13-14°C can withstand 1- to 4-hour respiratory arrest if their circulation is supported by cardiopulmonary by-pass, and that the by-pass does not impair instrumental learned behavior.

Summary. Fourteen lightly anesthetized (Nembutal) rats were cooled to a body temperature of 16°C and then connected to a pump oxygenator built especially for rats. Then the rats were cooled further to 14°C which induced respiratory arrest. The extracorporeal blood flow of the hypothermic rats was 28 ml/kg/min (70% of the total cardiac output), while the delivery of oxygen was 1.7 ml/kg/min (100% of the total oxygen consumption). One to four hours later the animals were rewarmed to a body temperature of 25°C and the cardiopulmonary by-pass was then discontinued. Thereafter the animals were rewarmed to 37°C. All rats survived this cooling whereas all control animals without by-pass died. One day later the experimental animals were placed in a self-stimulating box. The animals had been trained before the by-pass to press a lever which induced electrical stimulation of their hypothalamic "pleasure center." After the by-pass the rate of intracranial self-stimulation of all animals was the same as before by-pass. It is concluded that a 4-hour period of hypothermic cardiopulmonary by-pass with respiratory arrest does not induce any temporary or permanent neurological changes in rats as judged by their instrumental performance through intracranial self-stimulation.

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