

# A Method for Extirpation of the Pineal Gland in Albino Rats

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A method for extirpation of the pineal gland in albino rats and other rodents (*e. g.*, ground squirrels) is proposed. Epiphysectomy is carried out by resection of a fragment of the bone with the underlying pineal gland. Using this method, many animals can be operated within a short period; the method is reliable and simple, which recommends it for chronobiological studies.

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**Key Words:** *epiphysectomy; albino rats*

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The pineal gland is an endocrine organ, whose functional significance remains not sufficiently well studied. It seems that its role of "biological clock" is the main one, but the mechanism of its work is not quite clear. Study of the biological role of the pineal gland is based mainly on experimental approaches, such as creation of epiphyseal deficiency by removal of the gland and administration of its biologically active substances. Sometimes the effect of "functional epiphysectomy" is attained by exposing the animals to 24-h illumination, which causes disorders in biological rhythms of activities of epiphyseal enzymes and hence, disorders in biorhythms of production of its main hormones [8]. On the other hand, this method does not completely preclude the effects of bioactive epiphyseal substances. Electrocoagulation of the pineal glands cannot guarantee its complete destruction [6], and hence, surgical methods should be considered more reliable, due to which they are most widely used [7,9,10]. However, it is not possible to operate many animals within a short time by the known methods of surgical removal of the pineal gland. It is desirable to follow up at least two periods of biological rhythm shortly before sacrifice in biorhythmological studies of various body functions. These studies should be carried out on a considerable

number of animals, which have to be operated on within a short period. This necessitates further mastering of methods of epiphysectomy. In addition, the methods of epiphysectomy have not been described up to the present time.

Using our simple and reliable method of extirpation of the pineal gland in rats and other rodents (for example, ground squirrels), it is possible to operate up to 40-50 animals per day. All manipulations on animals were carried out in accordance with the Order of Ministry of Health of the USSR "On Humane Handling of Experimental Animals" (No. 755 of August 12, 1977).

## MATERIALS AND METHODS

Male albino rats were narcotized with sodium thiopental (50 mg/kg intraperitoneally). The head of the animal was fixed by the external acoustic meatus and the maxilla (Figs. 1, 2). The hairs were cut in the zone of incision and the skin was treated with ethanol. The skin was cut along the median line of the head (1.5 cm). After exposure of the skull vault, a hole (3 mm) was drilled in the occipital bone behind crossing of the saggittal and parieto-occipital suture and the dura mater was dissected.

The pineal gland was removed by resection of a fragment of the bone with the underlying pineal gland using special pliers (Fig. 3), made from technical flat pliers. The lower branch of the pliers has

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a conical punch with rounded edge. The thickness of the working part is 1.5 mm, width 3 mm. A groove depression with sharp edges is located on the upper surface of the punch for collecting the detached bone fragment. The upper branch in the anterior part (matrix) has a window to match the lower branch punch with a small gap, due to which the bones are not cleaved, but cut.

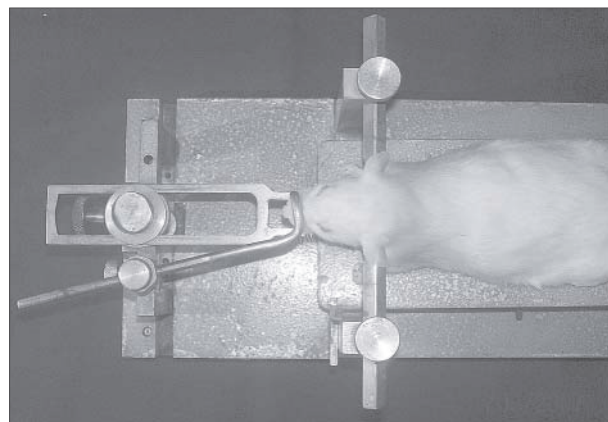
The bone was cut by lowering the upper branch matrix on the punch. The pliers lower branch matrix is inserted into the hole in the bone towards bone suture crossing between the lower surface of the bone and brain surface and a fragment of the bone together with fragment of the sinus and underlying pineal gland is cut. The removal of the pineal gland is controlled visually. Bleeding from venous sinus is arrested with a cotton tampon. In order to prevent subsequent adhesion between the connective tissue and brain tissue, wax paper is applied to the hole in the bone and the wound is sutured. The suture is disinfected with streptocide and mummy powder. The sutures are removed on day 7 after epiphysectomy.

## RESULTS

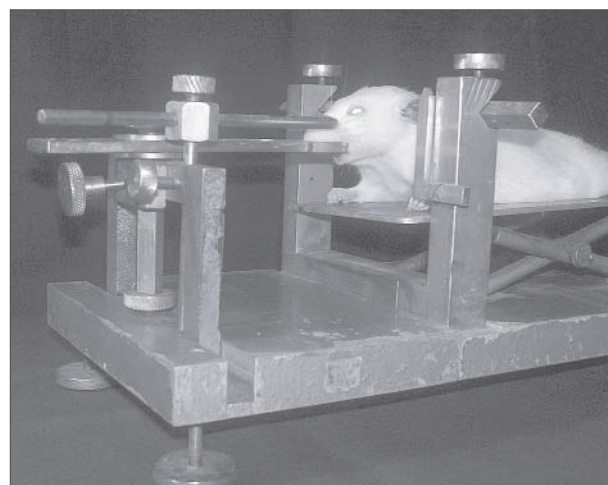
Mortality of animals from narcosis and blood loss during the first hours after surgery was 5%. Up to 30% animals died during subsequent 40 days because of cerebral circulation disorders and intestinal paresis associated with it. The flaw of the method is traumatic intervention and cerebral circulation impairment, which impedes evaluation of the studied functions during the first days after epiphys-ectomy. For higher productivity, the operation is carried out by two workers. The procedure of animal fixation and removal of the gland takes 10 min and is performed by one worker, while the other sutures the skin wound. We operated about 1500 animals by this method for evaluating the role of the pineal gland in regulation of circadian rhythm of proliferation and spermatogenesis [1-5].

The known surgical methods of epiphysectomy consist in removal of a part of the bone above the pineal gland without damaging the dura mater and venous sinuses, located under bone sutures, if possible. The latter can be rarely achieved. The pineal gland is then removed through incision in the dura mater by pincers or a tightened loop.

The bone fragment is removed by different methods. The most rapid method was proposed by V. Kh. Khavinson *et al.* [9,10] removing the bone flap with a 0.5 cm metal tube. However, this tube is to have sharp teeth at its end, which can lead to venous sinus damage in some cases. The known epiphysectomy methods are more sparing than our

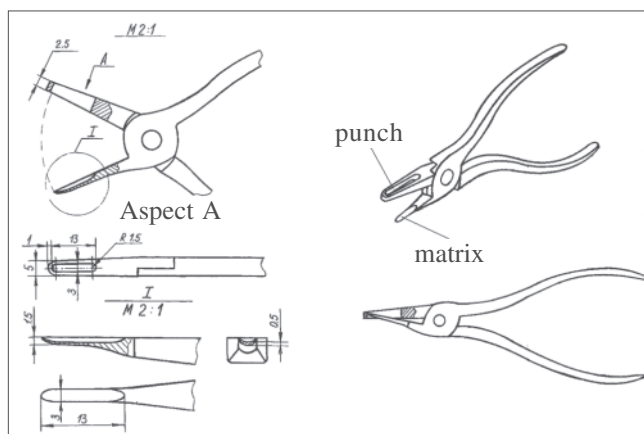


**Fig. 1.** Fixation of animal head by external acoustic meatuses.



**Fig. 2.** Fixation of animal head by the maxilla.

method, but the operation is longer, because the pineal gland is removed after removal of the bone fragment and bleeding arrest. In our method, the bone fragment with the underlying pineal gland are removed simultaneously, after which bleeding is arrested. During this procedure, epiphysectomy is carried out in the next animal.



**Fig. 3.** Design of pliers for epiphysectomy.

The material for studies of circadian biorhythms is as a rule collected 6-8 times a day over several days. The studies are carried out on many animals, in which epiphysectomy is to be performed within a short time. This is impossible to do by the known epiphysectomy methods. Using our method, we operated up to 50 animals per day and performed up to 500 operations within 2 weeks, 300 of these animals looking healthy on day 40 after epiphysectomy, when the material for the study was collected.

Hence, our method can be used in chronobiological studies.

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