AN EFFICIENT METHOD FOR
TRANSARURICULAR HYPOPHYSECTOMY IN RATS

By

Maroto Sato and Sakae Yoneda

ABSTRACT

A visible perforation at the correct site, (anterior section of the tense part of the tympanic membrane) was introduced instead of the blind procedure. In young rats, the utilization of a special needle with an orifice increased the probability of complete hypophysectomies as well as a high survival rate.

The anatomical position of the rat hypophysis near the thin wall of tympanum on an almost flat sella turcica offers many advantages which have been exploited by Koyama (1932) who introduced a simple method of transauricular hypophysectomy. In spite of undoubted superiority over the parapharyngeal (Smith 1930; Valle 1960, personal communication) and palatal (Thompson 1932) methods, this procedure is not generally accepted in view of the difficulties arising during the localization of the hypophysis, which depends on the skill of the operator.

Some technical modifications introduced by Tanaka (1955) and Falconi & Rossi (1964) help to overcome the objections to Koyama’s method. The present method guarantees a complete removal of the gland, as well as a high rate of survival.

MATERIAL AND METHOD

Hypophysectomy was performed both on young rats, less than 1 month old, and on grown animals about 300 g in weight. Operation for young animals: The rats are
anaesthetized with ether and then fixed on a board of special design (Fig. 1), by pushing a rubber ring through a short glass tube connected with the metal wire ring in which the superior incisor is located. The animal is placed in the abdominal position, its nose facing the operator. The animal is then turned through an angle of 90° to the left by the revolving sector. The tragus of the ear is lifted upward together with the cartilage with a pair of fine forceps. A small incision is practised horizontally in the tragus plane followed by a perpendicular cut so that 3 divisions of the tympanic membrane (flaccid parts, the tense part and the handle of the hammer) become visible in a small focus of light (Fig. 2). The anterior section of the tense part of the tympanic membrane, as indicated in Fig. 2, is pierced about 2 mm with a special needle (Fig. 3) with its cut end surface to the left, the upper part of the needle inclining about 15° towards the right and the opposite side of the operator from the perpendicular.

After perforating the tympanic membrane, the needle passes through the temporal bone and the point of the rock. If greater resistance is felt after this trajectory, the needle must have reached the sphenoidal bone. It should be introduced again with a slight move to the dorsal side. Thus the needle easily perforates the thin wall of the Cavum tympani without injuring to the N. Trigeminus. The animal with the needle inside, is then turned back 90° and the needle is introduced horizontally until its point begins to stick out through the right ear. In this way, the needle passes under the N. Trigeminus, on to the Capsula hypophysis and once more under the other N. Trigeminus until it perforates the tympanic membrane at the opposite side of the ear inflicting almost no injury to the neighbouring nervous tissue. The passing
Fig. 2.
Points of the reference for a puncture of the needle in the tympanic membrane.

- a ... Flacid part
- b ... Handle of hammer
- c ... Tense part
- X ... Puncture-point

Fig. 3.
Needles and metal marker.

- a ... mark
- b ... orifice
- c ... mandril
of the needle should be executed with great care since the success of the operation depends chiefly on this step. On this occasion, the hypophysis is sometimes pushed slightly forward or backward by the needle, but without any destruction of the tissue. Usually almost no resistance is felt if the needle is put through correctly, except for the occurrence of a slight haemorrhage. Once in position, the needle is turned through 180° around its longitudinal axis in order to bring the hole in direct contact with the hypophysis. A second identical needle should be placed parallel to the first one outside and over the head, in order to verify the position of the middle orifice, as a difference of 3 mm may mean either success or failure. A more convenient method consists in marking the middle line on the head with an ink-pencil and placing a special metal marker (Fig. 3) parallel to the needle until the mark coincides with the position of the sucking hole. The mandril is withdrawn and the syringe body, to the modified end of which the vacuum line of a water suction pump is connected, is placed on the needle. Slight left and right movements of the entire extracting apparatus (about 2-3 mm) with the hole facing downwards, followed by identical movements with the hole turned through 90° forward and afterwards backward, but carefully avoiding the upward position, help to loosen the gland and guarantee its complete extraction. The needle is then withdrawn and the animal released from the fixing board and returned to its cage.

Operation for older animals: A different type of fixing apparatus (Fig. 4) and another needle (Fig. 3) have to be used. The animal is fixed on its side with the dorsal part towards the operator, and the same operation is executed to disclose the 3 divisions, as mentioned above. The needle is introduced at an angle of about 60° through the anterior section of the tense part of the tympanic membrane until its point reaches the region of the hypophysis. Suction is then applied by a water suction pump.

![Fig. 4. Fixing apparatus for adult rats.](image-url)
RESULTS

Young rats, treated according to the method described above, gave a 90% rate of fully hypophysectomies and 90% survival rate in 600 animals. In adult rats, the complete removal of the hypophysis was achieved in 60% of the operated animals. The completeness of hypophysectomy was checked by careful examination of the hypophyseal region. In male rats completely hypophysectomized the testicles return to the inside of the abdomen 3 days after the operation. In female rats, examination of the vaginal smears can be used as a supplementary control.

DISCUSSION

In young rats, Koyama found the hypophysis directly under the middle line of the rat’s body, perpendicular to a line joining the openings of the auditory canals. As the rat grows older, the hypophysis becomes displaced towards the nose thus requiring a different method of transauricular withdrawal. As it is sometimes difficult to recognize the exact spot through which the needle should pass, the method of Koyama, who recommended a tentative feeling procedure, was substituted by direct vision. The whole ear lobe may be cut off but this operation is always accompanied by a considerable reduction in the chances of survival, probably due to the stress of pain. A small incision through the tragus with its cartilage, where relatively few nerve endings are found is sufficient to expose the tympanic membrane to direct inspection. The special construction of the needle allows a small stream of air to be sucked past the hole in front of the hypophysis, thus ensuring withdrawal by means of a slight pressure, according to the well-known principle of the water suction pump. Direct suction with a closed point needle is not always successful. A second hole through the other tympanum facilitates the entry of air and the withdrawal of the pituitary gland but increases mortality by causing haemorrhage of the submeninges. Pushing the needle directly through the other ear until it contacts the outside air, which can now enter via its open end, avoids all these difficulties and permits a safe removal of the hypophysis. The size of the needle is also critical as it must be of sufficient bore to allow the gland to pass through, but at the same time should be kept as thin as possible to keep the lesions small.

The main point of the proposed technique is the correct introduction and piercing motion of the needle as well as the location of the middle orifice in direct contact with the hypophysis. Any failure in ascertaining the correct placing of the needle increases the mortality caused by haemorrhage and damage of the neighbouring nervous system. When done correctly, at least
30 young rats can be hypophysectomized within an hour by one technician without help, with a 90% success. For older rats, where a straight perforation cannot be applied, the rate of successful hypophysectomy is around 60%.

Post-operative care as described by Koyama (1932) was used in our laboratory.

ACKNOWLEDGEMENTS

The authors express their gratitude to Prof. O. M. Calasans of the Dep. of Anatomy, School of Medicine, University of São Paulo, to Dr. Günter Höxter, Dr. E. H. L. Melo of the 1st Medical Clinics, Dra. Nina Krasnoschecoff of the Dep. of Obstetrics and Gynecology and to the carpenters of the Hospital das Clínicas, School of Medicine, University of São Paulo, for their cooperation.

REFERENCES

Falconi G. & Rossi G. L.: Endocrinology 74 (1964) 301.
Thompson K. W.: Endocrinology 16 (1932) 257.

Received on May 25th, 1965.