PRO EXPERIMENTIS

A new technique of pinealectomy for adult rats

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Summary. A new technique of pinealectomy for rats is described which brings the pineal into view of the experimenter facilitating its removal and also allows for completely parallel sham operations.

The position of the mammalian pineal gland makes a study of its function by surgical removal appear deceptively simple. But the many techniques described for pinealectomy all recognize the problem of brisk and often fatal hemorrhage from surrounding dural sinuses[2–6]. It is known that the pineal's sympathetic innervation from the superior cervical ganglia is essential for its function[7–9]. We have developed a new technique for pinealectomy on rats that, a) holds bleeding to a minimum, b) requires only brief duration of surgery, c) presents a clear view of the pineal gland at the operative site, facilitating its extirpation and decreasing the probability of accidental damage to adjacent neural structures, and d) provides the experimenter an opportunity to perform perfect parallel sham operations, making certain its innervation is not disturbed.

The heads of the anesthetized rats (sodium pentobarbital, 42.5 mg/ml; i.p.) are secured in a stereotaxic instrument (D. Kopf, Tujunga, CA) equipped with mouth bit and ear bars. After shaving the dorsum of the head, a longitudinal incision (ca. 1.75 cm) is made in the midline extending to the occipital ridge. The sagittal and lambdoid sutures are then exposed by scraping away the peroisteum to the temporal bone attachments of the temporalis muscles. Sequential cuts A, B, C, D (figure 1A) made with a dental drill using a No. 5 bone burr bit, isolate a rectangular section of the calvarium approximately 1.25 cm in its rostrocaudal extent and 0.75 cm in its mediolateral extent. The bone piece is then lifted off the underlying dura from its rostral edge exposing the superior sagittal vein (SSV), the transverse sinuses (TS) and the confluens sinuum (CS). Great care must be exercised in removing the bone piece to avoid causing hemorrhage from the sinuses below. With a No. 11 scalpel blade, cuts are made in the dura on the lateral edges of the SSV to the medial rostral edge of the TS. The SSV is now doubly ligated with 6–0 gauge surgical thread introduced under the SSV with a curved atraumatic needle, and resected. The resected portion should be minimal and markedly rostral to the CS facilitating reestablishment of adequate venous drainage following surgery (figure 1B). By reflecting the caudal portion of the ligated SSV posteriorly the gland is seen beneath the CS (figure 1C). The gland is approached from an anterior aspect and removed with a pair of fine curved watchmakers' forceps by grasping the base of the body. The procedure is completed by returning the reflected SSV to its original position, reapposing the skin, and closing the incision with two 11 mm Michel wound clips. The post-operative course was the same whether or not the bone flap was returned and complete recovery precluded the need for antibiotics. A comparison of weight gain between sham-operated and pinealectomized animals showed no significant difference. The entire procedure can be completed within 10–15 min. After more than 200 operations we have lost only 2 animals during or as a consequence of surgery.

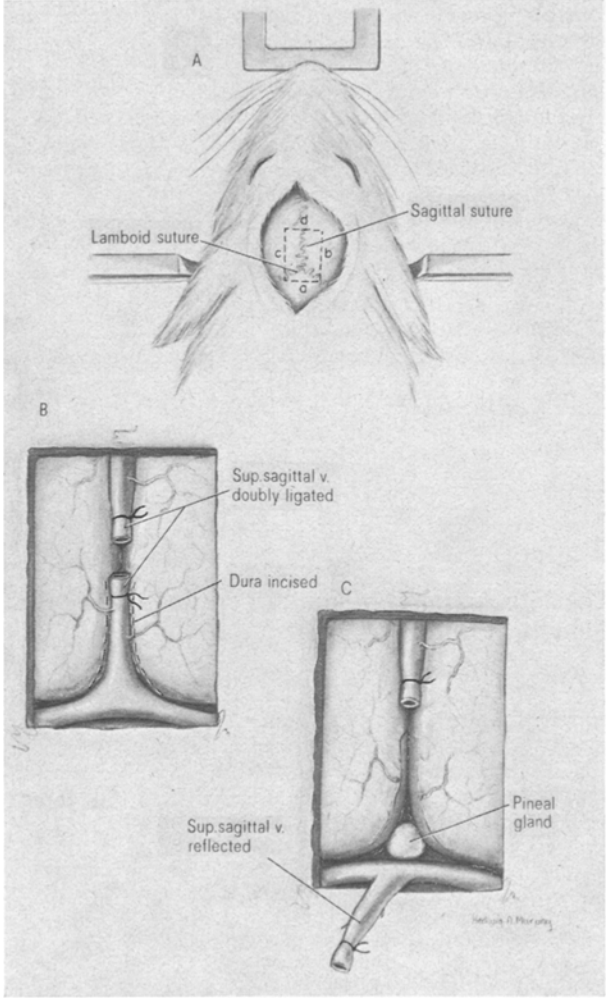

Fig. 1. A diagramatic representation of the stepwise surgical removal of the pineal gland in an adult rat.

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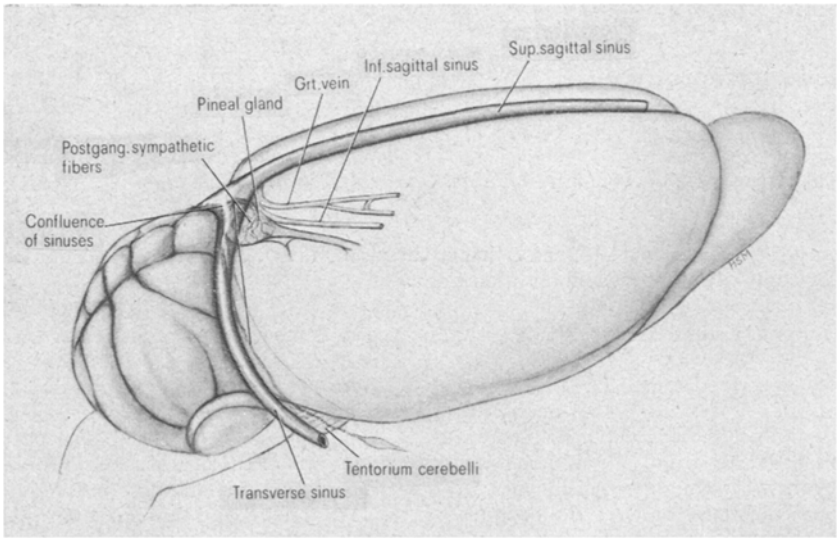

Fig. 2. A schematic drawing of the pineal gland's anatomical relations in the adult rat.

Our technique provides 3 distinct advantages for this type of surgery. First, an anterior approach is the most direct route to the gland. A posterior approach necessitates passing over the cerebellum and the corpora quadrigemina while a lateral approach involves pushing in a posteromedial pole of a cerebral hemisphere (figure 2). Secondly, owing to the location of the pineal in the center of the venous drainage system of the brain a certain amount of bleeding is inevitable with all of the previously described methods[10,11]. By reflecting the SSV posteriorly hemorrhage from the SSV and CS is obviated by effectively removing these venous channels from the surgical site. The pineal is also visible at this point making its extirpation simple and more certain. Bleeding when encountered, is usually from the inferior sagittal sinus and the venae cerebri magnae and can easily be contained by applying gently pressure with a cotton pledget or a piece of gauze. Third, and most importantly, with our method almost perfect sham operations may be performed. The post-ganglionic sympathetic fibres reach the pineal bilaterally having ascended up the carotid plexus and on the tentorium cerebelli. One method of pinealectomy described involves doubly ligating and resecting 1 of the 2 TS as well as the SSV. This method almost certainly effects a uni-lateral sympathectomy. With our technique the operated controls are placed under identical surgical trauma, but the innervation remains undisturbed. Thus a comparison of pinealectomized animals and operated controls following surgery measures the effect of removing the pineal and not of the surgical procedure.

In summary an investigation of pineal function by pinealectomy requires a procedure that recognizes the anatomical relations of the gland, the importance of its sympathetic input and a method that is quick, allowing the researcher an opportunity to work with a large number of animals. Our new technique was designed to meet these criteria and thus provide researchers in pineal physiology an important tool in studying the activity of this neuroendocrine gland.

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