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AN IMPROVED TECHNIQUE
FOR TRANSAURICULAR HYPOPHYSECTOMY AND
AUTOTRANSPLANT OF ISOLATED PIECES OF
PARS INTERMEDIA TISSUE IN
THE EVACUATED PITUITARY CAPSULE OF THE RAT

By
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ABSTRACT
An improved technique for transauricular hypophysectomy in rats, weighing 120–300 g, is described. This method can be used for transplantation studies, which is demonstrated by transplanting pars intermedia tissue into the evacuated pituitary capsule. Our technique has been carried out, successfully, in various laboratories. The success, however, depends upon the skill and care of the investigator.

The parapharyngeal route for hypophysectomy in rats, is a very common approach according to a survey done by Falconi & Rossi (1964a). However, this approach of hypophysectomy is apparently not very useful for transplantation into the evacuated pituitary capsule. Hence, we chose Koyama’s method of transauricular hypophysectomy in rats (Koyama 1931, 1962), with some changes, for autotransplantation in the pituitary capsule. Koyama’s method, although easier, has not gained popularity on account of certain drawbacks. For example, according to Zarrow et al. (1964), the hypophysis often comes out in pieces by Koyama’s method of hypophysectomy. Hence, it is difficult to judge if the hypophysectomy is complete or incomplete. For this reason, Falconi & Rossi (1964a,b), Sato & Yoneda (1966) and Pellet et al. (1969)

This study was supported by MRC of Canada Grant No MA-5160.
have further modified transauricular hypophysectomy procedure for rats and mice. *Falconi & Rossi* (1964a,b) have also described a method of transplantation of pituitary tissue in the evacuated pituitary capsule of hypophysectomized animals.

Since we were not successful with the hypophysectomy techniques described either by *Koyama* (1931, 1962) or by *Falconi & Rossi* (1964a,b), we decided to modify their technique insofar as the size of the needles and their adjustments were concerned, to obtain the best results. These investigators (*Koyama* 1962; *Falconi & Rossi* 1964a,b; *Zarrow et al.* 1964) have not given much importance to the size of the open tip of the needle.

The kit we have designed consists of a simple syringe with a hypodermic needle accurately designed by us, for hypophysectomy, and a set of small gauge needles for transplantation. In this paper, we shall describe details of the procedure for transauricular hypophysectomy as well as auto-transplantation of isolated pieces of pars intermedia tissue into the evacuated pituitary capsule of the rat.

**MATERIAL AND METHODS**

The hypophysectomy kit (Fig. 1a–f) comprises a 2 ml syringe (B-D Yale, Becton, Dickinson and Company, USA) and a suitable hypodermic needle selected according to the body weight of the rat (Table 1). If the weight of the animal ranges between 120–200 g, a 16 gauge B-D needle is used and if the weight ranges between 200–300 g, a 14 gauge needle is used. For the needles about 35 mm in length, a long plastic tubing (*Pellet et al.* 1969) or a rubber tubing is attached at the base of the needle, so that the exposed length of the needle is about 15 mm for the 120–200 g range and 18–19 mm for the 200–300 g range. Alternatively, a small rubber or plastic band is fixed at the base of the needle (Fig. 1a, at the arrow mark) and the extra length of the needle is cut off. The length of the open tip of the needle is adjusted to about 3 mm for 120–200 g range and about 4 mm for 200–300 g range (Fig. 1b), as shown in Table 1. It is essential that the area at the open end of the hypophysectomy needle is less by 1.0–1.5 mm than the actual size of the pituitary (see marked area in Figs. 1b and 6; and Table 1). We have noted that if the tip is smaller, then a part of the pituitary is excised and retained in the pituitary capsule, and hence the hypophysectomy is not complete. If the open tip is too big, then either it pierces through the pituitary capsule or the suction is not sufficient to remove the entire pituitary. These two points are important for the removal of the hypophysis in one piece. The open tip of the hypophysectomy needle also needs to be sharp and strong so that it can easily cut through the hard bones of the adult rats.

In order to obtain an assessment of the size of the needle, we have found that it is better to try the prepared needle in rats within the given range of the body weight (Table 1). After trial hypophysectomy, the needle should be left in the skull of the animal and the brain exposed, to find out exactly how far the needle has reached inside the pituitary capsule. Final adjustments on the length of the needle can then be made accordingly. This improves the accuracy of future hypophysectomies.

For hypophysectomy, the needle is introduced into the pituitary capsule of the rat.
Transauricular hypophysectomy and tissue transplantation kit for 200–300 g rat.

Fig. 1a. An 18 gauge B-D Yale transplantation needle is introduced into the hypophysectomy needle. The arrow mark shown indicates where to fit a rubber band to adjust the length of the hypophysectomy needle.

Fig. 1b. The tip in Fig. 1a is magnified 2½ times, showing the tip arrangement of the transplantation needle in the hypophysectomy needle. The measurements given are for rats weighing about 200–300 g.

Fig. 1c. A 2 ml B-D syringe with a needle for hypophysectomy.

Fig. 1d. A 0.25 ml B-D syringe fitted with an 18 gauge B-D Yale needle for transplantation.

Fig. 1e–f. The fine forceps used for isolation of pars intermedia tissues.

with care, so that the tip of the needle does not pierce through the other side of the capsule. If this does happen, it is not harmful for the hypophysectomy, but for transplantation, since the transplanted material comes out from the opening on the other side of the capsule.
Table 1.
Syringe needle size and dimensions, for rats of different sizes, for hypophysectomy and transplantation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Needle size (gauge)</th>
<th>Needle diameter (mm)</th>
<th>Needle tip length (mm)</th>
<th>Length of needle open tip (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>outside</td>
<td>inside</td>
<td></td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>120–200</td>
<td>16</td>
<td>1.6</td>
<td>1.2</td>
<td>9.0</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>200–300</td>
<td>14</td>
<td>2.0</td>
<td>1.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Transplantation</td>
<td>120–200</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Transplantation</td>
<td>200–300</td>
<td>18</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* This measurement is only for the type of needle which is shown in Fig. 1 a, b, c.

For transplantation of the tissue, a narrower hypodermic needle (B-D Yale) is selected (Fig. 1 d). The tip of the needle is removed by a grinder and the tip and length of the transplantation needle are so selected for size that the transplantation needle just fits into the hypophysectomy needle and is about 1 mm shorter in length as shown in Fig. 1 a, b; and Table 1. If any needle other than a B-D Yale is used, then the needle diameter, length and its open tip have to be manipulated carefully according to the body weight of the animal (Table 1). We found that, with a proper sized needle, as shown in Table 1, transplantation is accurate and success is 100%.

The procedure for hypophysectomy and autotransplant that we found to be most useful in rats, is as follows: A proper sized hypophysectomy needle is fitted to a 2 ml (B-D Yale) syringe as shown in Fig. 1 c. About 0.5 ml of sterilized saline is taken up in the syringe in order to take out the pituitary in the solution. A water suction pump used by Koyama (1962), Falconi & Rossi (1964a) and Sato & Yoneda (1966), was not found necessary by us. Sufficient suction develops in the 2 ml syringe, which enables one to detach and suck out the hypophysis. If sufficient suction is not produced in the syringe, application of a little grease to the plunger obtains the desired effect.

Rats are anaesthetized with ether or by an intraperitoneal injection of nembutal. The anaesthetized animal is placed flat on the table, its head held with the left hand and the needle is then introduced slowly with the right hand, into the external auricular opening at an angle of 45° as shown in Fig. 2. The surface of the needle faces towards the upper roof of the skull. The puncture of the ear drum is noted by a slight sound. The needle is then pushed carefully further into the bony canal of the ear which offers slight resistance. At this stage, the syringe is introduced forward and a little downwards, towards the other ear. More resistance is felt when the needle hits the hard bone of the preoptic capsule. The needle is now pushed slightly, so that its tip enters the diaphragma sella and comes just below the pituitary gland. The needle is then turned clockwise at 180°, so that its open tip faces the neural lobe, i.e. just above the pituitary gland (Fig. 6). In most of the cases, a little blood appears

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Figs. 2-5.

Transauricular hypophysectomy and transplantation of pars intermedia in the evacuated pituitary capsule of rat.

Fig. 2. Insertion of hypophysectomy needle into the ear of a rat by a 2 ml syringe.

Fig. 3. Suction of the pituitary in the syringe.

Fig. 4. Insertion of the transplantation syringe 0.25 ml, through the hypophysectomy needle in the ear.

Fig. 5. Transplantation of the tissues in the evacuated pituitary capsule by injection.

in the syringe at this stage, which in a way confirms that the proper area has been reached. The pituitary along with about 1 ml blood, is now sucked into the syringe by pulling the plunger (Fig. 3).

For autotransplantation of the pars intermedia, the hypophysectomy needle is left in the ear (Fig. 4) and the syringe is taken out. The type of needle shown in Figs. 1 a, b, c, holds better than the B-D Yale needle and fits firmly in the bony canal and the pinna of the ear. The pituitary is transferred to a Petri dish containing sterilized saline. If the material is pinkish, it is probably the pituitary; but if it is whitish, it is part of the brain. If a bony part gets stuck at the tip of the needle while cutting through the hard bone, the pituitary generally comes out in many pieces. In that case, it is best to reject such an animal because it is quite likely that small pieces of pituitary may be left in the capsule. After several successful operations, the open tip of the needle may get damaged, in which case, the pituitary gets cut into pieces. This can be avoided by carefully regrinding the needle tip to a smooth surface.

For transplantation of the pars intermedia back into the pituitary capsule, the pars intermedia is excised and separated from the pituitary as follows: The neuro-intermediate lobe is pulled out with a fine stainless steel forceps (Dumont & Fils, Switzerland)
land, Inox Nos. 5 and 7, as shown in Figs. 1 e, f). The pars nervosa always comes out along with the pars intermedia. Small pieces of pars intermedia are separated under microscope by slowly teasing the neuro-intermediate lobe. All the small isolated pieces of the pars intermedia are taken and placed on a slide in a single drop of saline. These pieces are examined under the microscope and all of the pieces are accommodated in the transplantation needle (refer Table 1 for size of the needle), fitted to a 0.25 ml syringe with 0.05 ml of saline (Fig. 1 d).

The transplantation needle holding the pars intermedia is introduced carefully into the hypophysectomy needle (Fig. 4). The transplantation needle will not move any further once it reaches the tip of the hypophysectomy needle. The syringe plunger is now gently pushed so that the material enters the empty pituitary capsule. No more than 0.05 ml saline need be injected (Fig. 5). Both the needles along with the syringe are now taken out and the syringe and the needle are checked to verify the quantity of the material that has entered the pituitary capsule.

This type of transplantation is not advisable in chronic hypophysectomized rats, because the tissue will not enter in the collapsed pituitary capsule once the hypophysectomy needle is taken out and the chances of success are less. With this technique, it is possible to transplant a tissue or one can introduce solid or semi solid hormone pellets or drugs into the pituitary capsule, with or without evacuation of the pituitary.

Post-operative care is simple. Each of the operated animal is kept in a separate cage in an airconditioned room (24–25°C), with one bottle of 0.9% saline and another bottle of tap water for drinking, until the time the animals are sacrificed. Up to 3–4 days after the operation, the animals are given bread soaked in 10% sugar and milk, as well as their routine food of Purina Lab Chow. For a few days, the animals prefer a liquid diet. The mortality rate is low with this post-operative care.

Fig. 6.

Schematic drawing to show the skull of an adult rat. The needle has been introduced into the pituitary capsule (PC). The outer sheath rests against the auricular canal, and the free tip of the needle faces the neural lobe (NL) of the pituitary gland when it is turned through 180°.

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Fig. 7.
Effect of hypophysectomy and transplantation on water and saline consumption of 1–6 rats. The lined zone indicates the average water and saline consumption of control rats.

RESULTS

The success of hypophysectomy was evaluated by measuring water and saline consumption, body and organ weights and vaginal smears. The success of transplantation was evaluated by gross examination of the hypophyseal region at autopsy and histological study of the pars intermedia.

1. Water and saline consumption

The effects of hypophysectomy and pars intermedia transplantation on the combined consumption of tap water and 0.9% saline per 100 g body weight per 24 h are shown in Fig. 7. In general, fluid intake was significantly higher in all the operated rats, for about 14 days after the operation. Thereafter, the fluid intake fell markedly and at the time of sacrifice, 45 days after the operation, it was almost normal (Fig. 7). Our data on fluid intake in these experiments are similar to that of the hypophysectomized rats of Kawashima et al. (1966). From these data, it can be briefly concluded that the release of neurosecretion might have gradually stepped up 14 days after hypophysectomy, due to the reorganization of the neural stump into a mini pituitary along with the growing pars intermedia tissue, so that, as the mini pituitary started functioning, the fluid intake gradually starts decreasing. Other histophysiological
Table 2.
Effect of hypophysectomy and pars intermedia transplantation on body weight (g) and weights of some organs (mg) per 100 g body weight\(^a\).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Body weight</th>
<th></th>
<th>Adrenal</th>
<th>Ovary</th>
<th>Uterus</th>
<th>Thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal rats</td>
<td>6</td>
<td>225 ± 4.1</td>
<td>235 ± 6.1(^b)</td>
<td>30.6 ± 2.45</td>
<td>32.4 ± 2.80</td>
<td>259 ± 32.65</td>
<td>8.2 ± 0.69</td>
</tr>
<tr>
<td>Rats with hypophysectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI transplantation</td>
<td>* 6</td>
<td>284 ± 2.8</td>
<td>260 ± 4.3</td>
<td>9.1 ± 0.56(^c)</td>
<td>13.3 ± 2.15(^c)</td>
<td>49.1 ± 4.86(^c)</td>
<td>5.7 ± 0.74(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Relative weight of the body, adrenals, ovaries, uteri and thyroids were calculated separately for each animal, and from their figures a variance series was described and the mean relative weight for each group calculated.

\(^b\) Mean ± standard error of the mean.

Statistical significance compared to normals.  \(^c\) \(P < 0.001\).  \(^d\) \(P < 0.05\).
and physiological details of the mini pituitary have already been dealt with in short communications by Naik (e.g., 1974).

2. Body weights

The body weights were recorded periodically. They showed an average reduction of 24 g (dropped from average 284 to 260 g), after the hypophysectomy and transplantation (Table 2).

Forty-five days after the operation, 9 female Holtzman rats with hypophysectomy and transplantation were sacrificed by decapitation. Their body weights, water and saline intakes and vaginal smears were recorded before decapitation. Fluid intake and organ weights tables were prepared in only 6 of the experimental animals, otherwise all the 9 animals were studied in this experiment.

3. Organ weights

Weights of thyroids, adrenals, ovaries and uteri were recorded after sacrifice. The thyroid weight was the least affected, but the adrenals, ovaries and uterine weights were markedly reduced (see the statistical significance in Table 2).

4. Vaginal smear study

The vaginal smear was recorded daily after the operation, until the time when the animals reached a persistent dioestrus. Once this phase was reached, the smear was studied 3 times a week in order to check their cycles. All the rats showed leukocytes (dioestrous phase) of the vagina, a few days after the operation. They maintained this condition until they were sacrificed 45 days after the operation.

5. Gross examination of the hypophyseal region at autopsy

The autopsy examination revealed that a small pituitary piece, beaded in some cases, was attached to the reorganizing neural stump. The beaded structure of the hypophysis may be due to the growth of several groups of pars intermedia cells in the pituitary capsule. The blood vessels present in the pituitary seemed to be intact with the reorganizing neural stump.

6. Brief histological study of the transplanted pars intermedia

Immediately after the animals were sacrificed, the pars intermedia was dissected out along with the pituitary capsule intact with the hypothalamus. The material was fixed for 24 h in Bouin's fixative and paraffin blocks were prepared as per routine. Serial 6 μm sections were cut and stained with
Fig. 8.
Sagittal section of the pituitary capsule 45 days after the pars intermedia transplant into the rat. The pituitary capsule (PC) has nicely maintained its entire shape (R - rostral zone; C - caudal zone). Many large and small patches of pars intermedia growth scattered in the pituitary capsule are marked by arrows. Quite a lot of highly stained colloidal material (CO) is also seen. Note the connective tissue and blood vessels in the central part of the pituitary capsule. The 6 μm section is stained with Gomori's aldehyde fuchsin.

Gomori's aldehyde fuchsin. All the experimental rats with isolated small pieces of pars intermedia graft showed a growth of pars intermedia in the pituitary capsule which was covered with connective tissue and maintained an oval shape (Fig. 8). Some animals showed pars intermedia growth very close to the regenerating neural stump. In some of the pituitary capsules, large and small, round or oval patches of growing pars intermedia were seen (Fig. 8). These regenerating pars intermedia cells were mostly polygonal or oval, light glandular cells. ACTH cells were numerous near the regenerating neural stump whereas, they became scarce away from the neural stump. The remainder of the capsule was filled with colloid like substance and it took up a deep orange G stain. The blood vessels and connective tissue were seen in between the growing islands of pars intermedia cells.

Our results along with the measurements of certain parameters for hypophysectomy (Table 1), indicate that this experimental design is successful for preparing hypophysectomized rats for laboratory experiments. This technique has already been carried out with great success in several laboratories.
ACKNOWLEDGMENTS

The authors would like to express their deep appreciation to Prof. Belá Halász, Department of Anatomy, Faculty of Medicine, Budapest, Hungary, for his help and advice in this investigation.

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Received on August 13th, 1974.