

ENDOCRINE/EXOCRINE MODIFICATION PROCEDURES



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Introduction

This reference paper describes Charles River's surgical capabilities for endocrine/exocrine modification procedures offered by its Research Models and Surgical Services group. Most of the procedures can be conducted on rats, neonatal rats, and mice. For each procedure, this paper describes pre-operative and surgical procedures along with postoperative care. For more detailed information or questions on any of the services offered, please contact Charles River Technical Services at 1-800-338-9680.

Preoperative Preparation

Unlike humans, rodents do not normally regurgitate. Hence, except for certain procedures such as the pancreatotomy, there is no need to fast animals to prevent the potential aspiration of stomach contents during anesthesia and surgery. However, a period of withholding food lasting less than twelve hours does help to ensure consistent absorption of intraperitoneally administered injectable anesthetics. Since it does not affect anesthetic absorption, water is never withheld. Plus, the state of hydration could have adverse effects on the level of anesthesia and survival postoperatively.

Following anesthesia of the animals, the operative site is prepared by shaving. The skin surrounding the incision site is then decontaminated by the application of povidone iodine solution, followed by 70% isopropyl alcohol.

Incision Closure

The method of wound closure is dependent upon the size and location of the incision. If muscles are not transected, but rather bluntly dissected, separate closure by layers may not be necessary. In the case of extensive incisions, it may be necessary to close by tissue layers using an interrupted suture pattern. Skin is generally closed by wound clips, but can also be closed either with non-absorbable suture or tissue adhesives (e.g., cyanomethacrylate).

General Postoperative Care

Unless otherwise specified in the procedure, the following postoperative care is practiced at Charles River. (See our Surgical Capabilities Reference Paper Vol. 13 No. 1 for more detailed information.)

The animal should be provided supplemental heat to maintain core temperature, taking care to monitor the temperature closely to ensure that burns do not occur. At Charles River, one of two procedures may be used for heat supplementation - either a 60 watt incandescent bulb placed 12-18 inches from the animal, or a bedded plastic cage placed on a temperature-controlled heating pad. Room temperature is maintained between 74-80°F. The skin incision should be monitored for any signs of infection.

Unless otherwise specified in the procedure, animals are maintained on standard rodent diets post-operatively. Water is given ad libitum. A minimum of 24 hours of postoperative recovery is recommended before shipping the animal.

Analgesia

All rats and mice that have undergone any surgical procedure require analgesic administration. Buprenorphine or Flunixin meglumine is injected subcutaneously. Buprenorphine is the standard default analgesic. Flunixin meglumine is substituted upon request.

Standard Equipment and Supplies

The following is a list of equipment and supplies generally used in the procedures described in this reference paper, as well as during preoperative and postoperative care.

- sterile saline
- 70% isopropyl alcohol
- povidone iodine surgical scrub
- #10 or #11 scalpel blade and #3 scalpel handle
- auto clips (9 mm or 7 mm)
- auto clip applier (9 mm or 7 mm)
- auto clip removal forceps
- appropriate anesthetic
- appropriate analgesia
- sterile gauze squares (2 x 2 inch)

- trephine instrument (custom-made for Charles River)
- dental drill
- fine suction tip with fingertip control and vacuum system
- 20, 23, 25, 26, and 27 ½ gauge needles
- 4-0, 5-0, and 6-0 absorbable and silk suture material with curved atraumatic needle
- curved iris forceps
- angled, sharp, delicate non-toothed microsurgical forceps (3-1/2")
- halsted mosquito forceps (3-1/2")
- straight mouse-toothed thumb forceps (3-1/2")
- custom-made curved stainless steel wire retractors
- cotton-tip applicator (3" long)
- dissecting microscope (up to 15X magnification)
- 3-1/2" straight, sharp, castroviejo microdissecting spring scissors
- 4" straight, sharp microdissecting scissors
- 5" straight, blunt metzenbaum dissecting scissors
- 1, 3, 5 and 10 mL syringes
- surgical drape, size according to procedure and species
- 60 watt incandescent bulb (placed 12-18" from animal) or bedded plastic cage on temperature-controlled heating pad for supplemental heat
- electric hair clippers with fine shaving blade
- petri dish (5" diameter)

ADRENALECTOMY, ADRENAL MEDULLECTOMY

An adrenalectomy is the surgical removal of one or both adrenal glands. An adrenal medullectomy is the surgical removal of the adrenal medullae. The adrenal glands are small, round, orange/pink-colored endocrine organs located near the kidney. Each adrenal gland is composed of an inner core-like medulla and an outer bark-like cortex. These glands produce steroid-based glucocorticoid and mineralocorticoid hormones, as well as epinephrine. An animal can survive normally without the adrenal medullae, but sodium levels must be monitored and supplemented either with dietary sodium or mineralocorticoids for the animal to survive when the adrenal cortex is also removed.

Preoperative Procedure

The animal is weighed and anesthetized with appropriate anesthetic (please refer to our Surgical Capabilities Reference Paper, Vol. 13. No. 1, for more details on anesthesia). The hair on the back of the lumbar area is clipped. The animal is then placed in ventral recumbency and the surgical site prepared as described in the preoperative section of this paper.

Surgical Procedure

A 1 cm dorsal midline skin incision is made using a #10 blade on a #3 scalpel handle at the level of the 1st to 3rd lumbar vertebra. The muscle wall is entered with a pair of halsted mosquito forceps 1.5 cm lateral to the spine on each side. The left adrenal is located lateral and cranial to the spleen and is embedded in adipose tissue. The right adrenal gland is located cranial to the kidney. In young animals, very little fat is present to obscure the adrenal glands and, thus, they are very easily seen. However, some careful dissection may be necessary in older animals, where the presence of fat commonly conceals the glands.

Once identified, the adrenal glands, together with the surrounding fat pad, are exteriorized by grasping the periadrenal fat with a pair of straight mouse-toothed thumb forceps. The adrenal glands are then excised by blunt dissection using two pairs of mouse-toothed forceps. The gland itself must not be grasped. This can cause pieces of the adrenal to be broken off and to possibly re-implant itself in the abdominal cavity and regain function. Please note that use of this surgical approach will miss accessory adrenal gland nodules if they are present. The incidence of these nodules is minimal and strain dependent.

Postoperative Care

In addition to normal postoperative care described in the above section, adrenalectomized animals also require additional care. For long-term survival, adrenalectomized animals must be given postoperative treatment to replace the loss of sodium that occurs as a result of this operation. This may sometimes include the administration of corticosteroids, however, most often, adrenalectomized animals are given normal physiological saline (0.85-0.9% NaCl) to drink ad libitum in order to maintain sodium levels. Other sources of water should not be presented.

Saline supplementation is critical during transportation to ensure the survival of the animal. Charles River gives every

adrenalectomized animal sterile saline, injected subcutaneously prior to shipment to the customer. Depending on animal size, rats are given 5-10 mL and mice are given 2-3 mL.

It is important to understand that certain inbred strains of rats and mice may not tolerate this surgical procedure as well as outbred or hybrid strains. One may see an increased incidence of perioperative mortality as well as less tolerance of shipping stress, which may result in morbidity and mortality during transit.

ADRENAL MEDULLECTOMY

The adrenal medulla secretes two hormones, epinephrine and norepinephrine. Adrenal medullectomy removes the medulla from the cortex, leaving the capsule plus a layer of glomerulus cells. Enough cortical tissue remains or regenerates from these to meet the requirements for cortical hormones.

Preoperative Procedures

See the adrenalectomy preoperative procedures previously described.

Surgical Procedure

A 1 cm dorsal midline skin incision is made using a #10 blade on a #3 scalpel handle at the level of the 1st to 3rd lumbar vertebra. The muscle wall is entered with a pair of halsted mosquito forceps 1.5 cm lateral to the spine on each side. The adrenal glands are located as described above in the adrenalectomy procedure. The adrenal glands are exteriorized by grasping the periadrenal fat with a pair of straight, mouse-toothed thumb forceps.

A small incision is made on the adrenal capsule with 3-1/2" straight, sharp castroviejo microdissecting spring scissors, and the medulla is gently squeezed out with a pair of straight, atraumatic thumb forceps. The medulla pops easily out of the capsule. The capsule and the remaining attached fat pad are returned into the abdominal cavity. The skin incision is then closed with wound clips.

Postoperative Care

In addition to the standard postoperative care described above, normal drinking water should be administered following this surgical procedure.

HYPOPHYSECTOMY (PARAPHARYNGEAL APPROACH)

A hypophysectomy is the surgical removal of the pituitary gland. Often considered the “master gland” of the endocrine system, the pituitary gland (or hypophysis) is a small, pink, oval endocrine organ found at the base of the brain. It produces several hormones that directly or indirectly impact most basic body functions, including growth.

Preoperative Procedures

The animal is weighed and anesthetized with appropriate anesthetic. The ventral neck region is clipped and shaved. The animal is placed in dorsal recumbency with the tail toward the surgeon and the surgical site prepared as previously described.

Surgical Procedure

A 1.5 cm ventral midline incision is made along the length of the neck up to the point of the mandible using a #11 blade on a #3 scalpel handle. The strap muscles (hyoid) of the neck are bluntly dissected to reveal the base of the cranium. Using a custom-made trephine (for rats) or a dental drill (for mice), a small hole is made in the cranium in order to visualize the pituitary gland. The gland is covered with a fine membrane, which is torn with a 26 gauge needle to free the gland. The gland is then gently removed with aspiration using a fine suction tip and the incision closed.

Postoperative Care

In addition to normal postoperative care, the animal is given 5% glucose or sucrose to drink ad libitum. A minimum of 72 hours of recovery is recommended before shipping the animal. In order to identify animals with incomplete removal of the pituitary gland, it is recommended that a comparison be done of preoperative body weight and postoperative body weight 5-7 days after surgery. Animals with complete removal of the pituitary will have minimal change in these values.

It is important to understand that certain inbred strains of rats and mice may not tolerate this surgical procedure as well as outbred or hybrid strains. One may see an increased incidence of perioperative mortality as well as less tolerance of shipping stress, which may result in morbidity and mortality during transit.

PITUITARY GLAND TRANSPLANT TO RENAL CAPSULE

This procedure is normally done to induce hyperprolactin, a condition involving the overproduction of the hormone prolactin, which stimulates lactation in mammary glands.

Preoperative Procedures

The same preoperative procedures are followed here as are followed for a hypophysectomy.

Surgical Procedure

For auto-transplantation (transplanting the pituitary gland from an animal into its own renal capsule), the following procedures are used. The animal is hypophysectomized and the pituitary gland recovered. A paralumbar laparotomy is then performed to expose one kidney. A 20 gauge needle loaded with pieces of the pituitary gland is inserted into the renal capsule and the pieces of the gland injected beneath the renal capsule. The abdominal muscle incisions are closed with 3-0 or 4-0 absorbable suture using interrupted sutures, and the paralumbar and neck skin incisions are closed with 9 mm auto clips.

For hetero-transplantation, a pituitary gland obtained from another animal (donor) is implanted into a different animal's (recipient) renal capsule using the above procedure.

Postoperative Care

The same postoperative care is used for this procedure as is for the hypophysectomy.

PANCREATECTOMY

A pancreatectomy is the surgical removal of all or part of the pancreas. The pancreas is a large gland that secretes digestive enzymes and insulin. In practice, total pancreatectomies are seldom done. Partial pancreatectomies (usually leaving one-third of the organ intact) represent over 95% of pancreatectomy procedures done.

Preoperative Procedures

For this specific procedure, animals are fasted for 24 hours prior to the surgery. The abdomen is shaved from the xiphoid cartilage to the symphysis pubis.

The animal is placed in dorsal recumbency with the tail toward the surgeon and the surgical site prepared as usual.

Surgical Procedure

The pancreas is located in the mesentery and is bordered by the stomach, spleen, and duodenum. It is pink in color, diffuse, and closely follows the first one-third of the duodenum.

Removal of the pancreas in rats is difficult. The procedure can be divided anatomically into three segments. Removal of the gastrosplenic and duodenal portions removes about 95% of the pancreas and is considered to be a partial pancreatectomy. Removal of the biliary portion as well removes 99.5% of the organ and is considered to be a total pancreatectomy.

Access to the pancreas is gained through a 3.5 cm midline abdominal incision extending about two-thirds the length of the abdomen posterior to the xiphoid cartilage using a #10 blade on a #3 scalpel handle. Once identified, the pancreas is carefully elevated using a 3" cotton-tip applicator and placed on the surgeon's gloved finger. A cotton-tip applicator is then used to tease the pancreas free of its mesenteric attachments. This type of blunt dissection is used in order to avoid damaging the blood vessels supplying the pancreas. The muscle incision is closed with 3-0 absorbable suture material using a continuous interlocking pattern. The skin incision is closed with 9 mm auto clips.

Postoperative Care

Total pancreatectomies require insulin to keep the animal alive. Blood glucose levels must be tracked carefully and insulin dosages must be adjusted based on blood glucose levels. Such maintenance is very labor intensive and contributes to the difficulty of conducting total pancreatectomies in rodents.

Partially pancreatectomized animals are given standard rodent diets without supplementation while recovering at Charles River.

THYROID-PARATHYROIDECTOMY

In the rodent, the parathyroid glands are intimately embedded in the thyroid gland, therefore removal of the thyroid gland also removes the parathyroid gland. The glands are responsible for producing a parahormone that

regulates calcium metabolism, which is essential for maintaining normal muscle contraction as well as many cellular ion transport mechanisms.

Preoperative Procedures

Animals are weighed and anesthetized with appropriate anesthetic. The hair on the ventral neck area is clipped. The animal is then placed in dorsal recumbency and the area is surgically prepared.

Surgical Procedure

A 1.5 cm ventral midline skin incision is made along the length of the neck from its base just below the point of the mandible using a #10 blade on a #3 scalpel handle. The two halves of the sternohyoid muscle are separated and retracted laterally using stainless steel retractors. The paired thyroid glands are small pink organs, one on either side of the trachea just below the larynx and extending caudally along the first four tracheal rings. They are connected across the ventral aspect of the trachea by a thin band of tissue, the isthmus. The minute parathyroid glands are embedded in the anterior part of each thyroid gland. Using fine-angled microdissecting forceps, gently tear the isthmus connecting the two lobes of the thyroid glands. With the forceps and blunt dissection, remove both halves of the thyroid gland, taking special care on the right side to avoid damage of the recurrent laryngeal nerve, which may cross the dorsal surface of the gland.

Postoperative Care

In addition to the postoperative care described earlier, calcium supplementation (1% calcium chloride or 1% calcium gluconate) in the drinking water is recommended following this surgical procedure. This supplementation is generally given for 7-10 days to help maintain calcium homeostasis during the post-recovery period.

The most common complication of a parathyroidectomy is damage of the recurrent laryngeal nerve. Damaging this nerve can result in paralysis of the larynx, causing respiratory impairment that can be fatal.

THYROIDECTOMY

The paired thyroid glands are small pink organs, one on either side of the trachea just below the larynx. Thyroid glands synthesize and secrete thyroid hormones T3 and T4. These hormones regulate the development of the skeletal

and central nervous system. T3 and T4 also play a role in oxidative, carbohydrate, lipid, and nitrogen metabolism.

Preoperative Procedures

Animals are weighed and anesthetized with appropriate anesthetic. The hair on the ventral neck area is clipped. The animal is then placed in dorsal recumbency with the tail or head towards the surgeon and the area is sterilized as previously described.

Surgical Procedure

A 1.5 cm ventral midline skin incision is made along the length of the neck from its base just below the point of the mandible using a #10 blade on a #3 scalpel handle. The two halves of the sternohyoid muscle are separated and retracted laterally using curved stainless steel retractors. The parathyroid glands are removed from the surface of the thyroid using fine-angled microdissecting forceps. The glands are placed in a sterile petri dish with saline. The thyroid glands are then removed as described previously. The parathyroids are then placed on either side of the trachea positioned where the thyroid glands have been extracted. The sternohyoid muscles are placed over the parathyroids and the skin incision closed with 9 mm auto clips for rats and 7 mm auto clips for mice or neonatal rats.

Postoperative Care

In addition to the standard postoperative care described above, normal drinking water is recommended following this surgical procedure.

PINEALECTOMY

The pineal gland is a very small (1-2 mm), round, translucent organ located on top of the brain. It functions to secrete melatonin and may help regulate the pituitary.

Preoperative Procedures

The animal should be weighed and anesthetized. The hair on the head is clipped. The animal is placed in ventral recumbency with the head toward the surgeon's left and the surgical site scrubbed.

Surgical Procedure

A 1.5 cm dorsal midline sagittal skin incision is made to expose the skull using a #11 blade on a #3 scalpel handle. The 5 mm diameter plate of bone is removed

using a custom-designed trephine or a dental drill to cut the plate and expose the gland. The pineal gland is then removed by blunt dissection with a pair of forceps. The piece of bone is replaced (if a trephine is used), and the skin incision closed with 9 mm auto clips for rats and 7 mm auto clips for mice or neonatal rats.

Postoperative Care

No special postoperative care is needed other than routine care described earlier.

THYMECTOMY

The thymus gland is a bilobed, roughly heart-shaped structure located in the anterior portion of the chest cavity cranial to the heart and at the thoracic inlet. It participates in the functional development of the body's immune system.

Preoperative Procedure

Animals should be weighed and anesthetized. The hair on the chest and ventral neck area is clipped. The animal is placed in dorsal recumbency with the head toward the surgeon and the area prepared for surgery.

Surgical Procedure

Each lobe of the thymus is more or less separate, but held closely together by a connective tissue covering. Occasionally, when trying to remove the gland, it may separate, making it necessary to remove both parts individually. In older animals, care must be taken when separating the connective tissue, as it may have become tough.

A 2.5 cm midline incision is made in the skin from the base of the neck posteriorly over the thorax using a #11 blade on a #3 scalpel handle. The thorax is opened by transecting the first two ribs starting at the thoracic inlet to expose the thymus. The tissue is then retracted to completely expose the thymus. Care should be taken because of the close proximity to major blood vessels. The gland is removed using a cotton-tip applicator to gently tease/scrape the thymus from the incision. After the gland is removed, the chest is compressed using pressure between the thumb and forefinger to expel air from the thoracic cavity. During compression, the skin is closed with 9 mm auto clips for rats and 7 mm auto clips for mice or neonatal rats to

seal the thoracic cavity. It is important to close the thorax quickly to prevent and/or alleviate respiratory distress caused by the pneumothorax associated with this procedure.

Postoperative Care

Normal postoperative care is practiced.

THE CUSTOMER'S ROLE IN POSTOPERATIVE MANAGEMENT

Providing surgically-modified animals to the customer is a team effort requiring communication and follow-up between Charles River Laboratories and the customer.

Post-Shipment Animal Evaluation

Upon receipt of the animals at the customer's facility, the customer should thoroughly examine the animals for signs of any postoperative complications or clinical abnormalities. The animals should be given free access to food and water as soon as possible and placed in a temperature-controlled environment. They should be provided with a clean, bedded cage, which should be changed as frequently as required to ensure that the operative site does not become excessively moistened with contaminated fluids from soiled bedding.

Post-Shipment Nutrition

In the case of animals in which endocrine organs have been removed, some supplementation may be required in either the food or drinking water. Charles River Laboratories will provide the receiving institutions with the necessary information on which supplemental materials are required.

Any animal that appears to be dehydrated should first be given access to drinking water and observed for ingestion of water. Supplemental fluids can be given subcutaneously, but should be done on the basis of veterinary input.

Wound Care

In most cases, dressing of wounds and applications of local antiseptics or disinfectants is not required. Wounds are closed most commonly with auto clips. These should be removed 7-10 days following the surgery. By this time, there is generally enough tensile strength in the healing wound to ensure that all layers will remain closed. Special auto clip removal forceps are commercially available, although other surgical instruments can be used for this purpose.

Customer's Responsibility

Any abnormal occurrences with respect to the health of the animals or the success of the surgery should be conveyed to Charles River Laboratories. Please contact Charles River Laboratories' Technical Services Department at 1-800-338-9680.

IACUC

Charles River's Institutional Animal Care and Use Committee (IACUC) governs the entire surgical process, including any postoperative holding in Charles River facilities prior to shipment. The receiving institution's Animal Care and Use Committee, investigators, and animal care staff are responsible for the well-being of the animal subsequent to its arrival at their institution. Justification for use of surgically-modified animals, review of experimental protocols, authorization to order animals that are surgically modified from Charles River, and all aspects concerning the use of surgically-modified animals after they arrive at the institution are the responsibility of the receiving institution's IACUC.

The endocrine and exocrine modifications described in this paper are available in the following species:

| Procedure | Rat | Neonatal Rat | Mouse |
|---|-----|--------------|-------|
| Adrenalectomy | x | | x |
| Adrenal Medullectomy | x | | x |
| Hypophysectomy | x | x | x |
| Pancreatectomy | x | | |
| Thyroid-Parathyroidectomy | x | | x |
| Pinelectomy | x | | x |
| Pituitary Gland Transplant to Renal Capsule | x | | x |
| Thymectomy | x | x | x |
| Thyroidectomy | x | | x |

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