Common Surgical Procedures in Mice and Rats

Marcel Perret-Gentil
The University of Texas at San Antonio
marcel.perret@utsa.edu
LARC Main Page: vpr.utsa.edu/larc
LARC Training Page: http://goo.gl/rzR3zl

Procedures in this Presentation
• Partial and total splenectomy (dorsal and ventral approach)
• Ovariectomy
• Ovariohysterectomy
• Orchiectomy (scrotal and abdominal approach)
• Scrotal and abdominal vasectomy
• Adrenalectomy
• Nephrectomy
• Ureter ligation

Procedures in this Presentation
• Partial hepatectomy in mice (with and without gall bladder removal)
• Subdiaphragmatic vagotomy
• Pilooplasty secondary to subdiaphragmatic vagotomy
• Removal of sciatic nerve section
• Common carotid catheterization
• External jugular vein catheterization
• Femoral artery catheterization
• Femoral vein catheterization

Disclaimers
1. Images and videos associated with this presentation are for the purpose of demonstration of surgical techniques and are not intended to teach or demonstrate aseptic technique
2. As author of this presentation, I firmly advocate that survival surgery in rodents should be performed with meticulous attention to aseptic technique
3. Procedures shown in this presentation conducted as terminal procedures in anesthetized animals, under a protocol approved by the animal ethics committee at my institution

Before beginning...

Skin Closure

Surgical clips

Clips, clip applier & clip remover
Non absorbable, monofilament suture

Silk is not an appropriate suture for skin closure

Silk on Skin

- It is easy to handle making it the suture of choice of many investigators... however...
- Should not be used for skin closure for following reasons:
  - Produces undue local reaction and inflammatory response
  - It is braided and through its wicking action serves as a fomite to introduce microorganisms into the wound
  - These properties result in potential clinical and subclinical infections
  - As such, it is not consistent with sound principles of veterinary medicine

Approaching the Spleen

Dorsal & Ventral Approach

Dorsal Approach

~1 cm skin incision
- On animal's left side
- Parallel to 13th rib
- Dorsal extreme beginning just below the spinal muscle

Ventral Approach

- Make a 1-2 cm mid ventral skin incision with its extreme cranial end at the level of the stomach
- Abdomen is entered through the linea alba
- Spleen is identified below and to the left of the linea alba
- Exteriorize spleen
Partial Splenectomy (Biopsy)

Via a Ventral or Dorsal Approach

Spleen is accessed via a dorsal or ventral approach

1. Tie a ligature around the spleen arm to stem bleeding
   Do not include splenic vessels into ligature
2. Excise splenic tissue distal to ligature

Partial Splenectomy (Biopsy) in the Mouse – Video

Ventral Approach
3:08

Video – Partial Splenectomy

Total Splenectomy

Via a Ventral or Dorsal Approach
Spleen is accessed via a dorsal or ventral approach

1. Exteriorize spleen
2. Cut gastro-splenic ligament with scissors or cautery to separate spleen from stomach
3. Identify, isolate and ligate splenic vessels. In tiny mice careful cauterization of vessels without a ligature may be performed with caution

Cauterize or transect blood vessels distal to ligature

In rats (especially large ones) isolate individual blood vessel bundles and ligate

Isolating blood vessels

Ligate & Cut
Cautery alone works well in smaller animals (mice)

• Muscle layer is closed with absorbable suture
• Skin is closed with
  – Monofilament, non-absorbable suture in an interrupted fashion, or
  – Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to close small skin incisions or to reinforce (and provide a microbial barrier) larger incisions

Total Splenectomy in the Mouse Video
Ventral Approach
3:28

Video – Total Splenectomy

Ovariectomy

• Make a transverse or longitudinal dorsal incision ~ half distance between hump of back and level of knee with animal in ventral recumbency
• Transverse incision allows easier bilateral access to both ovaries through same incision
1. Identify white fatty tissue surrounding ovary
2. Identify ovary, fallopian tube under white fatty tissue
3. Retract ovaries with forceps

Cut or cauterize to remove ovary
With rats (specially if large) may place a ligature to minimize bleeding

Cut or penetrate and separate abdominal wall muscle fibers with tips of sharp scissors (iris scissors) to locate ovary
Alternative to Traditional Cautery

1. Submerge tips of hemostatic forceps in a hot bead sterilizer
2. Remove and immediately clamp tissue

• Muscle layer is closed with absorbable suture
• Skin is closed with
  – Monofilament, non-absorbable suture in an interrupted fashion, or
  – Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to close small skin incisions or to reinforce (and provide a microbial barrier) larger incisions

Mouse Ovariectomy Video

1:55

Ovariohysterectomy

Mouse & rat female reproductive track
1. Make a ventral midline skin incision with its extreme caudal end at level of the pubis (bladder)

2. To enter abdomen, linea alba is incised in same direction as skin incision

- Exteriorize uterus with its surrounding fat & tissues
  - If necessary move bladder to the side or empty it with a syringe & needle
- Identify uterine body, horns and ovaries

- Ovaries, uterine horns and uterine body are dissected free to separate from other tissues. In large rats, a ligature distal to ovary minimizes bleeding
- Cautery is useful to dissect tissues away from uterus

- Clamp uterine body or cervix with hemostatic forceps
- Place a ligature distal to hemostatic forceps

- Muscle layer is closed with absorbable suture
- Skin is closed with
  - Monofilament, non-absorbable suture in an interrupted fashion, or
  - Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to close small skin incisions or to reinforce (and provide a microbial barrier) larger incisions

Remove (excise) uterus along with ovaries proximal to hemostats with cautery, surgical blade or scissors
Mouse Ovariohysterectomy Video

1:58

Scrotal Orchiectomy

- Rats & mice have open (loose) inguinal canals. This allows free movement of testes between scrotum and abdomen.
- During scrotal castration, testes may need to be forced into scrotum. This may be done by exerting pressure on testes towards the scrotum in the caudal abdomen with fingers or Q-tips.

Incise scrotum on midline
Incise parietal tunica, avoiding cutting the vaginal tunica, which is intimately associated with & adhered to the testes.

In mice, use scissors.
A firm tug exteriorizes the testes

Pull until spermatic cord is exposed

Procedure is similar in mice & rats

Cauterize or ligate spermatic cord

Alternative to Standard Cauterization

1. Submerge hemostats in a hot bead sterilizer
2. Immediately clamp to seal and transect the spermatic cord

Repeat procedure on contralateral testis

Close scrotum (options)
- Monofilament, non-absorbable suture in an interrupted fashion
- Surgical Clips
- Surgical glue

Orchiectomy Video, Scrotal Approach
2:44
**Video – Scrotal Orchiectomy**

- Rats and mice have open inguinal canals, allowing free movement of testes between scrotum and abdomen
- Testis may need to be pushed from scrotum to lower abdomen towards. This may be done by exerting pressure on scrotum towards abdomen with fingers or Q-tips

**Abdominal Orchiectomy**

- The traditional abdominal castration makes a ventral midline incision close to the pubis, which can damage seminal vesicles while testis are being searched
- Suggested is a refinement to this technique in next slide

- A refined approach consists of making a ventral midline skin and muscle incision, starting at umbilicus & continuing caudally for a few millimeters
- Testis are pulled out through the incision and removal is performed as described under the scrotal orchiectomy

**Umbilicus**

- Exteriorize whitish fat that is related to the testis
- Pull this fat along with testis
Clamp below the testis ensuring no testicular tissue is trapped in the clamp

Cauterize between the testis & clamp

- Replace excess tissue back into abdomen
- Muscle layer is closed with absorbable suture
- Skin is closed with
  - Monofilament, non-absorbable suture in an interrupted fashion, or
  - Surgical Clips
  - Surgical skin glue (cyanoacrylate), which provides a microbial barrier

Vasectomy – Vas Deferens

Abdominal or Scrotal

Testis is exteriorized through the incision and closure as described under scrotal and abdominal orchiectomy

Vas deferens is relatively massive and easily visualized as a white/bright tubular structure with a blood vessel running alongside it

- Vas is also recognized as continuation of the epididymis
- Dissect out/isolate the vas deferens
Vas deferens is relatively massive and easily visualized as a white/bright tubular structure associated with the epididymis.

Excise ~0.5 cm section of each vas deferens with a blade, scissors or cautery with or without suture ligations.

Vasectomy Video in the Mouse

2:15

Adrenalectomy

A 1-2 cm (depending on size of animal) mid-dorsal (or slightly lateral) skin incision is made with its extreme cranial end at the level of the 13th rib.

Needle shows location of 13th rib.
A window is made through muscle fibers by entering muscle and separating fibers bluntly with sharp (iris) scissors.

- Muscle layer is closed with absorbable suture
- Skin is closed with
  - Monofilament, non-absorbable suture, or
  - Surgical clips

Nephrectomy

- Adrenal gland is located cranial and medial to kidney, embedded in fatty tissue
- Remove the gland intact with forceps without needing to cut

- Adrenal gland is closely associated (medial and cranial to kidney) with the kidney and should not be extracted or damaged during nephrectomy
- Adrenal gland is left intact in the body

- Right kidney is slightly cranial to the left kidney
- Right kidney is partially hidden under the right lobe of the liver
- Left side nephrectomy is often the preferred approach for unilateral nephrectomy
Ligate with 1 or 2 sutures and remove kidney

Second ligature more important the larger the animal is

Renal vessels and ureter are isolated

• Muscle layer is closed with absorbable suture
• Skin is closed with
  – Monofilament, non-absorbable suture in an interrupted fashion, or
  – Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to close small skin incisions or to reinforce (and provide a microbial barrier) larger incisions

Nephrectomy Video

2:29
Ureter Ligation

To simulate unilateral obstructive nephropathy

The kidney is exteriorized as described under nephrectomy session

Due to its translucid nature, ureter may be difficult to visualize

Ureter is localized as it leaves the renal pelvis posterior (caudal) to renal artery and vein
Ureter is ligated with 1 or 2 ligatures or with vessel clamps (for temporary occlusion) to simulate obstructive nephropathy.

- Muscle layer is closed with absorbable suture.
- Skin is closed with:
  - Monofilament, non-absorbable suture in an interrupted fashion, or
  - Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to close small skin incisions or to reinforce (and provide a microbial barrier) larger incisions.

**2/3 Partial Hepatectomy in Mice**

Two Methods

**Liver Anatomy**

Why is this a mouse & not a rat liver?

Rats lack a gall bladder.

In most strains of mice the relative weights of the respective lobes are constant.

Anatomy similar in rats, except rats lack a gall bladder.
Mammals can survive removal of up to 75% of total liver mass.
If > 75% is removed, remaining liver mass is not sufficient to maintain critical levels of blood glucose.
Removal of <1/3 will not elicit a generalized liver proliferative response.
Therefore, median and left lobe hepatectomy is considered ideal and results in removal of ~ 64-68% of total liver mass, a partial hepatectomy technique known as 2/3 Hepatectomy.
After resection of 2/3 of the liver, remaining hepatocytes undergo one or two replication rounds without complications related to hypoglycemia.
2/3 hepatectomy is therefore a preferred method for studying dynamics of liver regeneration.

Surgical Technique

• Dorsal pressure helps with liver exposure

- Paper clip
- Xiphoid process

• Recommended retraction to expose liver with paper clips, rubber band and needles

- Midline incision is extended beyond (cranial to) xiphoid process
- Xiphoid process is retracted with rubber band to further expose liver

• Recommended retraction to expose liver with paper clips, rubber band and needles

- Midline incision is extended beyond (cranial to) xiphoid process
- Xiphoid process is retracted with rubber band to further expose liver

Q-tips soaked in saline are useful tools for moving lobes

Before placing ligatures at the base of the lobes, cut various ligaments as illustrated here in the middle lobe.
Partial Hepatectomy – Method #1

**Two** ligating sutures

**Gall Bladder Removed**

- Gall bladder is removed with median lobe
- This technique should not be used in C57 mice, as it may result in death in 7 days or less
- 1st ligature placed at base of left lobe
- 2nd ligature placed somewhere between the 2 dashed lines
- Ligating too too close to vena cava, may result in stenosis of the vena cava

1st ligature placed at base left lobe

2nd ligature, on right & left median lobules include gall bladder

Vena cava is observed at base of median lobe

Visualizing vena cava in method #1 is key to avoid placing ligature to close to it and to avoid vena cava stenosis

After ligation use sharp scissors to excise (remove) liver lobe distal to ligature
Partial Hepatectomy Video
Method #1, Gall Bladder Removed
3:48

Partial Hepatectomy – Method #2

*Three* ligating sutures
Gall Bladder Left in Place

- 1st ligature at base of left lobe
- 2nd ligature around base of right median lobe
- 3rd ligature around base of left median lobe
After ligation, distal to ligatures, lobes are excised with sharp scissors.

- Muscle layer (linea alba) is closed with absorbable suture.
- Skin is closed with:
  - Monofilament, non-absorbable suture in an interrupted fashion, or
  - Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to reinforce closure (and provide a microbial barrier).

Subdiaphragmatic Vagotomy

- A mid-ventral skin incision is made with its extreme cranial end at the level of xiphoid process.
- Abdomen is entered through linea alba in same direction as skin incision.

Both anterior and posterior branches of the vagus nerve are excised.
Esophagus & both branches of vagus (anterior & posterior) are identified caudal to diaphragm. With delicate blunt dissection, separate both vagal branches away from esophagus.

Dissection/separation of vagus branches from esophagus can be done with micro-scissors, fine tip forceps or by hydrodissection. Hydrodissection is done by inserting a lacrimal cannula attached to a 1 ml syringe between esophagus & vagus branch, followed by injection of small volume of saline to separate tissues.

Cánula lacrimal

Dissection/separation of vagus branches from esophagus can be done with micro-scissors, fine tip forceps or by hydrodissection. Hydrodissection is done by inserting a lacrimal cannula attached to a 1 ml syringe between esophagus & vagus branch, followed by injection of small volume of saline to separate tissues.

Each vagal branch is individually cut or ligated depending on study objectives. Vagotomy in mice requires high magnification.

Pyloroplasty as Adjunct Surgery to Subdiaphragmatic Vagotomy

The Problem and the Solution

- The pyloric sphincter controls output of food from the stomach into the duodenum
- **Problem:** Vagotomy results in pylorus inability to allow stomach emptying into duodenum
- **Solution:** Pyloroplasty widens the pylorus to allow passage of food into the duodenum
Pyloric sphincter
Duodenum
Antrum

Longitudinal incision is made across & into pylorus
Incision is closed transversally

Results

Before pyloroplasty
After pyloroplasty
Emptying of stomach is now possible

Suture technique of pyloric incision method #1:
• Suture: Monofilament, absorbable
• Layers: 1 layer-closure, inverted
• Instruments: Microsurgical

Suture technique of pyloric incision method #2 (preferred):
• Suture: Monofilament, absorbable
• Layers: 1 layer-closure, inverted
• Instruments: Microsurgical

Horizontal mattress pattern
Longitudinal incision

Pyloric sphincter

Distal end of longitudinal incision

Proximal end of longitudinal incision

Retracted suture on both sides stretch pylorus transversally before beginning closure of pylorus

Sciatic Nerve Excision

Sciatic nerve runs caudal parallel to femur

Femur is identified by palpating knee and coxofemoral joint
• Longitudinal incision:
  • ~3-5 mm caudal to femur
  • Most proximal end at level of coxofemoral joint (or greater trochanter)
• Bluntly dissect through intermuscular white band

With sharp pointed scissors (e.g., iris scissors) penetrate white fibrous band, separating muscle bellies without cutting muscle
Continue separation & dissection until white sciatic nerve becomes visible

• Dissect & elevate sciatic nerve with fine mosquito hemostats
• Cut sciatic nerve
• Approximate muscles with absorbable suture in a simple continuous pattern
• Close skin with clips or non absorbable, monofilament interrupted suture

Common Carotid Cannulation
This method is used to introduce catheter or telemetry device into artery

Place a syringe hub or similar object (size depends on animal size) under neck to help expose the blood vessel
• Surgeon position is cranial to nose or caudal to animal depending on surgeon’s preference (I prefer cranial to nose)
• Make a ~2-3 cm mid ventral incision over trachea (or just lateral to it)

• Common carotid is identified after separating muscles that run parallel to trachea

• When arteries are manipulated, they easily go into spasm and collapse, complicating the introduction of the catheter (cannula) into the artery
• Suggestions to minimize spasm & collapse
  1. Prevent the artery from drying ... Keep moist with warm saline at all times
  2. Minimize handling the artery ... If manipulating artery is necessary, grasp the tissue adjacent or above the artery rather than the artery itself
  3. 1-2 drops of 2% lidocaine over artery helps minimize spasms

• Artery is carefully separated from vagus nerve
• Avoid damaging recurrent laryngeal nerve, which runs alongside the trachea (medial to carotid & vagus)
• Note in image above: Great care is taken to minimize grasping artery, but rather grasp tissue surrounding it
• Place 2 ligatures distal & proximal to where catheter will be introduced. 3 ligatures can be placed for added security
• Distal suture (towards head) is tightened to ligate artery
• Proximal suture (towards heart) is kept lose

With forceps place tension on proximal suture (closest to heart) to kink artery
This avoids excessive bleeding when artery is entered (incised)

Alternative to kinking:
Artery can be occluded with vascular clamps proximal (closer to heart) to artery incision point

• ~50% of artery’s diameter is incised
• Arterial incision should be made close to distal suture (closest to head)

Suggestion to create a catheter introducer
Suggestion to create a catheter introducer:

- With needle driver, bend hypodermic needle at 90-120° as illustrated 2 images on right
- This creates a channel to help slide catheter under cut lumen of the needle point
- Point of needle penetrates blood vessel & catheter is introduced under needle

Instead of kinking artery with proximal suture (closest to heart), artery occlusion can be made with a vascular clamp. This prevents excessive bleeding when artery is incised.

Although using either method will lead to some bleeding, use of vascular clamps will lead to less bleeding.

Catheter is inserted directing towards the heart.
Suggestion: Catheter forceps (with internal grooves) are useful tools to grasp & introduce catheter into artery or vein, while minimizing damage to catheter

- After introducing catheter, tie proximal suture over artery and catheter
- Tie distal suture around exteriorized catheter

Common Carotid Artery Catheterization Video
4:24

External Jugular Vein Catheterization
External jugular is visualized as it disappears under pectoral muscle.

Skin incision points of reference:
Direction of skin incision will be a straight line somewhere between pectoral muscle & a point just (slightly) medial to the angle of the jaw.

Note: Orientation of jugulars is not exact. Cartoon is for illustration purposes and not to describe exact anatomical landmarks.
Place a syringe hub or similar object (size depends on animal size) under neck to help expose the blood vessel

**Surgeon position is cranial to nose**
- Make a 1.5-2.5 cm incision
- Incision started at level of pectoral muscle & directed towards angle of jaw (or slightly medial to it)

Once located, dissect external jugular free of surrounding tissue

**Place 2 sutures under vein**

**Cut here**

**Suggestion for passing suture under blood vessel**
- Insert suture through hub of small/sterile pipette
- Force tip of pipette under vessel while rotating pipette back & forth about its longitudinal axis
- Once through the "other" side, grasp suture with forceps
- While grasping suture on "other" side, remove pipette & leave suture in place

**Most cranial (towards head) ligature is tied and knotted**
- Most caudal (towards heart) ligature is left loose without tying (for now)
~25% of vein's diameter is incised

Catheter is introduced directed towards (in the direction of) the heart

- After introducing catheter, tie caudal (towards heart) suture over vein and catheter
- Tie cranial (towards head) suture around exteriorized catheter

External Jugular Catheterization Video
6:41

Femoral Artery and Vein Catheterization
• Femoral Vein & Artery catheterization is performed following the same principles discussed earlier in this presentation for the common carotid and external jugular sections
• The difference is how to approach & manipulate the femoral vessels. See following slides for description of these differences
• Femoral Vein, Artery & Nerve run adjacent and parallel to each other. Their orientation from posterior to anterior they run in this order, VAN (Vein, Artery & Nerve)

Immediately after making the incision, a body of fat (fat pad) is encountered, which is retracted away or excised to visualize femoral Vein, Artery & Nerve lying right underneath this body of fat

• Incision is made longitudinal or perpendicular to the femoral furrow according to the surgeon's preference
• Incision is made on the medial surface of the thigh over the areas traversed by the femoral Vein, Artery & Nerve, with its most extreme cranial end (if incision is longitudinal) at the level of the inguinal ligament

• Incision should expose inguinal area, evident by visualization of a white band of tissue (inguinal ligament)
• Vein, Artery & Nerve disappear proximally under the inguinal ligament as they enter into the abdominal cavity

• Incision should expose inguinal area, evident by visualizing a white band of tissue (inguinal ligament)
• Vein, Artery & Nerve disappear proximally under the inguinal ligament as they enter the abdominal cavity

Separation of Vein, Artery & Nerve can be done with micro-scissors, fine tip forceps or hydrodissection
Hydrodissection is performed by inserting a lacrimal cannula attached to a 1 ml syringe between vessels, followed by injection of small volume of saline to separate structures.

Femoral Vein or Artery catheterization is performed following the same techniques described earlier in this presentation in the common carotid and external jugular sections.

- Subcutaneous tissue is approximated with absorbable suture in a continuous pattern
- Skin is closed with
  - Monofilament, non-absorbable suture in an interrupted fashion, or
  - Surgical Clips

Surgical skin glue (cyanoacrylate) may be applied to reinforce (and provide a microbial barrier) the incision.

Femoral Artery Catheterization in the Rat Video
8:04
Femoral Vein Catheterization in the Rat Video

4:01

Marcel Perret-Gentil, DVM, MS
University Veterinarian & Director
Laboratory Animal Resources Center (LARC)
The University of Texas at San Antonio
San Antonio, Texas

marcel.perret@utsa.edu

LARC Main Page: vpr.utsa.edu/larc
Training Resources: http://goo.gl/rr3R3s