**Appendix:**

**Table 1.1: Summary of Pre and Post-Surgical Procedures**

|  |  |  |
| --- | --- | --- |
|  | **Telemetry Device Implantation** | **T3 Transection Surgery** |
| Prophylactic Treatments | * Animals are acclimated to special liquid diet (Ensure®) for 3 days pre-operatively. Regular chow is not withheld until 10 hours before surgery.
* Enrofloxacin (10mg/kg once/day) is administered for 3 days pre-operatively.
* Hard chow is removed 10 hours before surgery (usually ~ 10-11pm)
 | * Animals are acclimated to special liquid diet (Ensure®), apple & orange pieces for 3 days pre-operatively. Regular chow is not withheld until 10 hours before surgery.
* Enrofloxacin (10mg/kg once/day) is administered for 3 days pre-operatively.
* Hard chow is removed 10 hours before surgery (usually ~ 10-11pm)
 |
| Anaesthesia & Surgical Site Preparation | * Surgeons wear a surgical scrubs, cap, mask and sterile gloves. Surgical sponges and gauze are autoclaved prior to use. Surgical tools are sterilized in a double-wrapped surgical pack prior to the surgeries.
* Animals are kept on a water-circulating blanket maintained at 37°C under1.5% maintenance dose of isoflurane. Isoflurane induction is at 4%.
* Skin preparation with alternate applications of disinfectant with alternating swabs of chlorhexadine (Hibitane) and 70% ethanol (three times each), followed by provodine iodine (Betadine) are applied for at least one minute, and removed with 70% ethanol (each time with a newly opened alcohol wipe). Scrubs are made in concentric fashion beginning at the center of the incision line and circling outward (so as not to contaminate the area by “back/forth” movements). Sterile gauze is used and discarded between each application. This process is repeated three times before starting animal surgery.
* Buprenorphine (Temgesic; 0.02 mg/kg) is administered SC immediately before surgery.
* Ketoprofen (Anafen; 5 mg/kg) is administered subcutaneously (SC) prior to surgery to provide multimodal analgesia.
* Bupivicaine will be administered SC just before surgery to provide local line block
 | * Surgeons wear a surgical scrubs, cap, mask and sterile gloves. Surgical sponges and gauze are autoclaved prior to use. Surgical tools are sterilized in a double-wrapped surgical pack prior to the surgeries.
* Animals are anesthetized under an induction dose of isofluarna of 4%.
* Intraperitoneal (IP) injection of ketamine(35mg/kg)/dexmedetomidine(0.25mg/kg) “cocktail” is given to anesthetized animal.
* Animals are kept on a water-circulating blanket maintained at 37°C. A nose cone with oxygen and isoflurane should be available if necessary.
* Skin preparation with alternate applications of disinfectant with alternating swabs of chlorhexadine (Hibitane) and 70% ethanol (three times each), followed by provodine iodine (Betadine) are applied for at least one minute, and removed with 70% ethanol (each time with a newly opened alcohol wipe). Scrubs are made in concentric fashion beginning at the center of the incision line and circling outward (so as not to contaminate the area by “back/forth” movements). Sterile gauze is used and discarded between each application. This process is repeated three times before starting animal surgery.
* Bupivicaine will be administered SC just before surgery to provide local line block.
* Buprenorphine (Temgesic; 0.02 mg/kg) is administered SC at around the mid-phase of the surgery (just prior to actual transection of the spinal cord) to account for the effects ketamine/medetomidine cocktail fading toward the end of longer surgeries. The later administration of the buprenorphine also provides longer-lasting analgesia during the acute recovery phase once the animal is awake.
 |
| Animal Housing | * After telemetry implantation surgery, animals are housed in cages (10 ½” x 19” x 8”) covered with wood chip bedding. Cages are smaller in this case in order to fit on telemetry charging pads (essential for charging and data collection from transducers). Water bottles are fitted with longer curved sipper tubes in consideration of the depth of these single animal housing cages.
* Animals singly-housed on wood chip bedding only need cage changed once per week.
 | * After spinal cord transection rats exhibit paralysis of the hind limbs, and mobilized with use of their forelimbs. The motor function of forelimbs with T3 transection is completely intact; however, they lack hind limb mobility and trunk and tail support. To facilitate their movement in the cage, a rubber grid should be placed under rolled oat bedding. This allows rats to grasp the grid with the forepaws, and move readily in the cage to access food and water. The bedding will be changed daily to keep the rats dry. In our previous experiments, we have found that this set-up allows injured rats to remain dry and move about their cage easily within two days of injury. The rolled oat bedding is used for the first 7 days post-surgery as we switched to this bedding when previous studies revealed that if and when animals ingested the wood chip bedding, this caused digestive complications. At days 7 post-SCI, the oat bedding is replaced by their original wood chip bedding.
* Rolled oat cages must be changed once every 2 days minimum. Once animals are returned to wood chip bedding, cages are changed twice per week.
 |
| Animal Diet | * Animals have rodent chow withheld for the first day post-operatively. Ensure® meal replacement drink is provided in a dispenser. DietGel® is provided on the cage floor for 2-3 days post-operatively.
* Post-surgery, animals are again provided with DietGel®, Ensure® and 1cm pieces of apples and oranges for 24 hours after surgery. Beyond that 24 hour period, the special diet is continued for 6 days, in addition to the solid chow, allowing the animals to be “weaned” back onto only solid rodent diet.
* Regular chow (LabDiet 5001) is re-introduced on day 2 post-operatively.
* Ensure® drink is continued for a further 6 days after surgery.
 | * Fruits, cereal, Ensure® meal replacement, DietGel® and a water dish are placed inside the cage for the animal for the first 8-14 days, depending how quickly the animal recovers the ability to move around the cage well; and depending on how quickly he is gaining weight. After 14 days animals are checked daily but weights and other monitoring information is filled out only 3 days per week. After 14 days there should be no need for any special diet such as fruits and Ensure®; however, Rodent Diet should always be readily available on the cage floor. Also when necessary, depending on the distance between the cage floor and the water bottle sipping tubes, long or curved sipper tubes should be considered.
 |
| Post-operative Monitoring & Care, Analgesic Plan | * For the first 14 days post surgery, animals are monitored 3 times daily. The early morning check involves weighing, scoring the animal based on our detailed monitoring key, and recording all information. The evening check involves assessing signs of pain or discomfort, and administering the second dose of buprenorphine. After 14 days animals are monitored every other day. The categories included in the monitoring key by which the animals are assessed are: Body Weight, Physical Appearance, Behaviour/Activity, Defecation, Skin (including autotomy and tumours/cysts), Hydration and Breathing. From this assessment we generate a composite score. A composite score at or above 8 (or 4 in any one category) indicates **IT:** the need for Immediate Treatment. A composite score at or above 10 (or 5 for any one category) indicates **IE:** immediate euthanasia.
* 5mL of warmed lactated ringer’s solution (15-30ml/kg) will be injected subcutaneously for the first three days after injury and will continue as monitoring of hydration deems it necessary. We have found that 5mL of LRS once daily usually suffices. For animals that show signs of dehydration (skin tent, pale ears/mucus membranes) we administer 10mL daily and twice if necessary.
* Buprenorphine is administered twice daily for 3 days post-operatively; once at 8:00am, and again at 8:00pm. A mid-day dose (0.01mg/kg) is given around 2:00pm if signs of pain/distress are noted.
* Ketoprofen is administered once daily 3 days post-operatively (during morning assessment ~8:00am)
 | * For the first 14 days post surgery, animals are monitored three times daily; initial morning check is when monitoring records are filled out and animals are assessed and scored. Each animal is assessed in seven categories: Body Weight, Physical Appearance, Behaviour/Activity, Defecation, Skin (including autotomy and tumours/cysts), Hydration and Breathing. From this assessment we generate a composite score. A composite score at or above 8 (or 4 in any one category) indicates **IT:** the need for Immediate Treatment. A composite score at or above 10 (or 5 for any one category results in **IE:** immediate euthanasia. Using our monitoring system, scores for animals with T3 SCI and sham injury are generally similar by two weeks post-surgery. Signs of pain and distress in the animals that we look for in our lab protocol include hunched posture, vocalization during bladder care, porphyrin around eyes/nose, facial grimace or eye squint. For animals that show signs of dehydration (skin tent, pale ears/mucus membranes) we administer 10mL daily and twice if necessary.
* Buprenorphine is administered twice daily for 3 days post-operatively; once at 8:00am, and again at 8:00pm. A mid-day dose (0.01mg/kg) is given around 2:00pm if signs of pain/distress are noted.
* Ketoprofen is administered once daily 3 days post-operatively (during morning assessment ~8:00am)For the first 5-7 days (or for as long as necessary) bladders are checked and expressed 3 times per day; 7:00-8:00am, 2:00-3:00pm and 9:00-10:00pm. After 7 days the bladder checks are usually just to check for and remove seminal plugs.
 |