

## **Xu lab protocol and instructions**

### **Notes on mouse surgery, viral injection and animal care**

#### **Mouse Genetically modified rabies 0.5-1 ul per injection ICV Single injections**

Important Steps to Follow: List the specific sequence staff should follow to avoid hazard.

Receive rabies vaccination (All the personnel working with rabies are vaccinated as a precautionous measure)

Use a certified biosafety cabinet when preparing virus

Don required PPE

Perform an airflow check prior to experiment

Disinfect all surface areas and surgical instruments with 10% bleach or 70% ethanol.

Label animal cages with biohazard cards.

#### Husbandry

AAV and Rabies infected mice will be housed in ABSL 2+ room located in MedSci Vivarium room 124S. AAV infected mice can be handled by ULAR staff. Label all cages with Biohazard card (example on last page) stating AAV infected mice..

For the rabies infected mice the Xu laboratory staff will be responsible for the husbandry, not ULAR staff. The rabies infected mice should be set on a designated shelf away from AAV infected mice. Cages must be labeled with a biohazard animal cage card stating the mice have been infected with rabies virus.

Remove PPE when exiting and wash hands thoroughly

#### Euthanasia

The infected mice will be euthanized in Med Surg. II room 336. Please separate water bottles from cages. All dirty cages must be placed in a red biohazard bag and sent for autoclaving. Do the same with the water bottles. These bags must be sent to MedSci Vivarium for autoclaving prior to cage washing. All animal carcasses should be bagged and placed in designated cold room. All carcasses must be disposed of as pathological waste.

#### Surgery for viral injection

The animals will be anaesthetized under 1-3% isoflurane inhalation. Once they have deep anesthesia indicated by loss of responses to tail pinches, the head skin will be incised and reflected, a small craniotomy will be performed, and a glass injection pipette will be lowered into the targeted brain region, and the virus will be injected into mouse brains with certain stereotaxic coordinates by using a pressure injector (picospritzer II ). After injections, the glass pipette will be retracted, and the head skin closed with surgical staples or sutures, sealed with topical antibiotic and anesthetic, the animal will be put back to the cage. This procedure is relatively brief, and can be completed within 10 minutes. The animal will wake up in a few minutes after getting off the gas anesthesia. We will monitor animal health states postoperatively.

## **Xu lab protocol and instructions**

Similarly, when the animal is under isoflurane anaesthesia, the head skin will be incised and reflected, a small craniotomy performed, and a cannulation base will be mounted with non-toxic dental glue for the placement of a cannula injector.

### **Methods to Prevent Dehydration & Hypothermia**

If needed, small animal heating pads (35-40 °C) will be used to keep the animal warm during the surgery and recovery.

### **Surgical Endpoints**

The surgical endpoints are defined as the end of injections when the glass pipette is withdrawn and the head skin is sealed, the animal is off the stereotaxic frame.

### **Post-Operative Care and Analgesic Usage**

All animals will receive a single dose of Carprofen (5 mg/kg subcutaneous) during the injection surgery.

We will monitor animals to make sure they are fine and can move around in the cage.

We will check upon animals at least once the next day, and if needed once more in the first week after the surgery.

### **Clinical Signs and Symptoms**

*Describe the clinical signs and symptoms that may appear in the animals as a result of the experimental procedures or agents, including any associated pain, distress or discomfort.*

The in vivo injection is an invasive procedure, but the pain and distress will be generally relieved under anesthesia. Clinical signs may include seizures, weight loss, lethargy and wound infection. Animals will receive a single dose of Carprofen (5 mg/kg subcutaneous) right after the injection surgery. If necessary, we will apply topical antibiotics and anesthetic (NEOSPORIN ®+ Pain Relief Cream) at the wounds after the procedure.

### **Monitoring Parameter**

*Describe the monitoring parameters that will be used to assess pain, distress and discomfort in animals (i.e., signs, symptoms and species-specific behaviors).*

Hypoactivity, squeaking noises and shivering are a few monitoring parameters to assess pain, distress and discomfort in animals.

### **Management Plan**

*Describe the management plan that will be used to assess and treat pain, distress and discomfort in the animals,*

## **Xu lab protocol and instructions**

*including any special procedures that will be used (e.g., periodic weighing of animals) and any interventions that will be performed to relieve pain, distress and discomfort in the animals (e.g., analgesics, antibiotics, special housing/bedding, etc.).*

The pain and distress cause by the surgery will be generally relieved under anesthesia. Animals will receive a single dose of Carprofen (5 mg/kg subcutaneous) right after the surgery. If deemed necessary, we will also apply topical antibiotics and anesthetic (NEOSPORIN ®+ Pain Relief Cream) at the wounds after the procedure.

Given that viral injection is a relatively minor surgery and the survival schedule is about 1-3 weeks, we don't anticipate adverse body weight loss of the animal subjects. We will monitor animals to make sure they are fine and can move around in the cage right after the surgery. We will visually check the animals the next day, and look for hypoactivity, squeaking noises and shivering to assess pain, distress and discomfort in animals. Abnormal health status will be noted at the cage card and in the experimental book, and the lab personnel will consult with ULAR vets. If needed (when the animal shows signs of severe pains or distress, or is not healthy to survive), we will euthanize the animal before the scheduled survival time.

### **Frequency of Animal Monitoring**

We will monitor animals' activities right after the surgery and wait for them to wake up. Then put them into the home cage. The animals that have undergone craniotomy will be checked at least once a day for at least 3 days after surgery.

### **Documentation of Animal Monitoring**

*Describe how the monitoring of animals (e.g., daily observations, treatments performed by research staff) will be documented. Monitoring records should be kept in the animal room and must be readily available to ULAR veterinary staff and IACUC members at all times.*

Next day observation, and weekly observation by research staff. The related information or monitoring records will be written at the cage card and in the experimental book.