2. Dissecting rodents

2.1. Organization of the field laboratory

(All authors)

2.1.1. Processing site

The choice of a convenient processing site can be a difficult task, as there are several requirements. First of all, the laboratory should be set up in a secluded area, away from humans and domestic animals. Outdoor processing is an alternative if electricity and tap water are not needed (or available) and the weather is favorable. Water tanks can be used as well as an awning to cover the field laboratory from sun and rain. A restricted area can be created by placing a ribbon around the processing site to clearly limit the intrusion by non-authorized persons. Processing rodents indoors requires an isolated and ventilated space that can be easily and regularly cleaned.

Everybody entering the processing site should wear protective equipment, including gloves, mask and laboratory white coat.

Before processing the animals, traps containing animals should be stored in a ventilated area, preferably outside and downwind, to avoid the inhalation of aerosolized particles excreted by rodents. They should be protected from the sun and changing temperatures. Rodents should be adequately fed and provided with water (fruits and vegetable are perfect) if not processed immediately.

Garbage should be removed every day. Used needles should be kept separately in a disposal container and if possible treated by a regional hospital or laboratory in accordance with local regulations. Other waste can be burnt daily at the processing site.

2.1.2. Avoid handling live animals

Fieldworkers should avoid handling live animals due to the associated risks of disease transmission through bites, contact with contaminated urine, blood, etc. or inhalation of infected particles. However in some cases this may be inevitable, for example:

- morphological and taxonomic studies when the animal has to be released (when dissection is not required for medical studies). Measurements can be taken on a living animal: sizes and weight (the animal can be placed in a bag and weighted together). Also, a small piece of tissue (e.g. hairs, top of the ear) can be taken for genetic analysis.
- serological studies: it is possible to collect a small amount of blood from the tail arteries and veins or the orbital sinus (see chapter 2.5).
- population studies: animals can be tagged to estimate densities, demographic patterns, niche extent, etc.
- production of karyotypes: injections should be done on live animals before processing (see chapter 3.4).

For these purposes, live animals should be handled gently with care to minimize stress to the animal. Different techniques can be applied, making sure that the animal can breathe and does not suffer. Small-size rodents should be grasped by the scruff of the neck using the thumb and forefinger. The tail can be secured with fourth and fifth fingers or with the other hand. Larger rodents can be held by catching the neck between the thumb and index fingers, while the hand covers the abdomen and maintains the animal. Another method is to grasp the head between the index and middle fingers, while the thumb and ring fingers are placed under the elbows. Thus maintained, the animal is rolled over, resting on the back in the entire hand.

2.1.3. Preparation of tubes

Recommendations:

- *Prepare tubes before starting dissection for quicker and safer processing (Fig. 6).*
- *If two tubes for one organ are needed, mark one of the two (with a cross for example) to differentiate the two series. It will help when sorting the tubes while transferring them from the liquid nitrogen to the -80°C freezer.*

Preparation:

***Tubes**:*

- *Use screw cap cryogenic tubes to store in a liquid nitrogen tank in the field (and in a -80°C freezer in the laboratory).*
- *Use color caps to rapidly sort and identify the organs collected. We use blue caps for lungs, white caps for spleen, yellow caps for kidneys and red caps for blood.*

- *Use Eppendorf® tubes to store in a fridge or a -20°C freezer.*
- *Prefer tubes with a safe lock to prevent any undesired opening.*

Labels:

- *Label tubes to be stored in liquid nitrogen with the rodent identification number. Use a permanent marker (beware or different qualities of markers; make a preliminary test to make sure that the label will last in liquid nitrogen).*
- *Prepare stickers with the rodent identification number for tubes stored in a fridge or a -20°C freezer.*

Tags:

- *Prepare two tags with the rodent identification number: one to be fixed to the body, and one to keep with the digestive tract in ethanol in a Falcon tube. We use the "Dymo®" stickers as they last a long time in ethanol. We use a nylon fishing line to attach this tag to the leg of each animal or the cheeks if the skull is separated for morphometrics.*

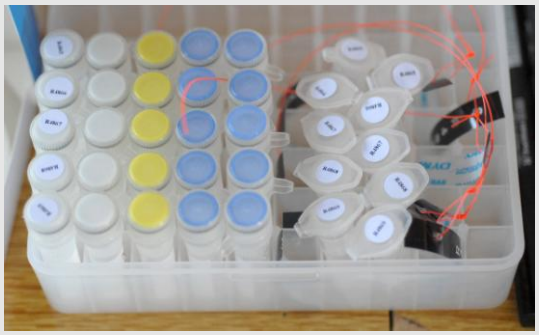

Figure 6: Tubes for dissection (Photo: Herbreteau V.)

Dissection table:

- *Prepare the processing table with all the equipment needed for each dissection.*
- *We recommend using a polystyrene board covered by a thinner plastic board to pin animals for a proper dissection. Such dissection boards can be easily disinfected and should be replaced regularly.*
- *Thoroughly clean the dissection board and tools with bleach then water then ethanol after each animal. Household bleach is a suitable disinfectant and can be easily found in remote areas. After cleaning with bleach, rinse with water to avoid the bleach corroding any metal surfaces (Fig. 7).*

Figure 7: Prepare Bleach, water and ethanol and replace regularly (Photo: Herbreteau V.)

2.2. Euthanasia

(Vincent Herbreteau, Yannick Chaval and Serge Morand)

Non-invasive techniques can be applied to several rodent studies (such as ecological surveys, morphological measurements, serology of pathogens, etc.). However, in certain cases, killing animals is necessary for research purposes: especially to keep voucher specimens for a reference collection and to isolate pathogens or parasites from internal organs. The necessity of sacrificing animals should be established under an approved research protocol guaranteeing the use of samples to answer the scientific objectives. When an animal is killed, a maximum of measurements, photographs, and samples should be taken for the purpose of the study but also for sharing with the scientific community if not required by the study.

Euthanasia is defined as the painless and stress-free killing of an animal. It is a sensitive question that recent guidelines have tried to clarify:

- AVMA (American Veterinary Medical Association Council on Research) Guidelines on Euthanasia (2007): http://www.avma.org/issues/animal_welfare/euthanasia.pdf
- CCAC (Canadian Council on Animal Care) guidelines on: euthanasia of animals used in science (2010): http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/PDFs/Euthanasia.pdf

We have tried to follow these guidelines for rodent studies and invite to read them carefully. We have summarized here the main recommendations.

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