LAB_061 Awake Photothrombotic Stroke Induction

I. OBJECTIVE

The purpose of this procedure is to ensure consistent production of photothrombosis stroke induction in mice with a previously implanted chronic cranial intact-bone window as per SOP LAB_0XY. The intact-bone window is a non-invasive procedure where the skull is exposed, and transparent dental cement is used to enhance the semitransparent nature of the mouse skull. In addition, a head bar is glued at the back of the head (on the skull) to allow for head-fixation. No special handling is required, and mice are housed together.

NB: The use of (*) indicates this statement is dependent on the facility procedures

II. COMMENTS / RECOMMENDATIONS

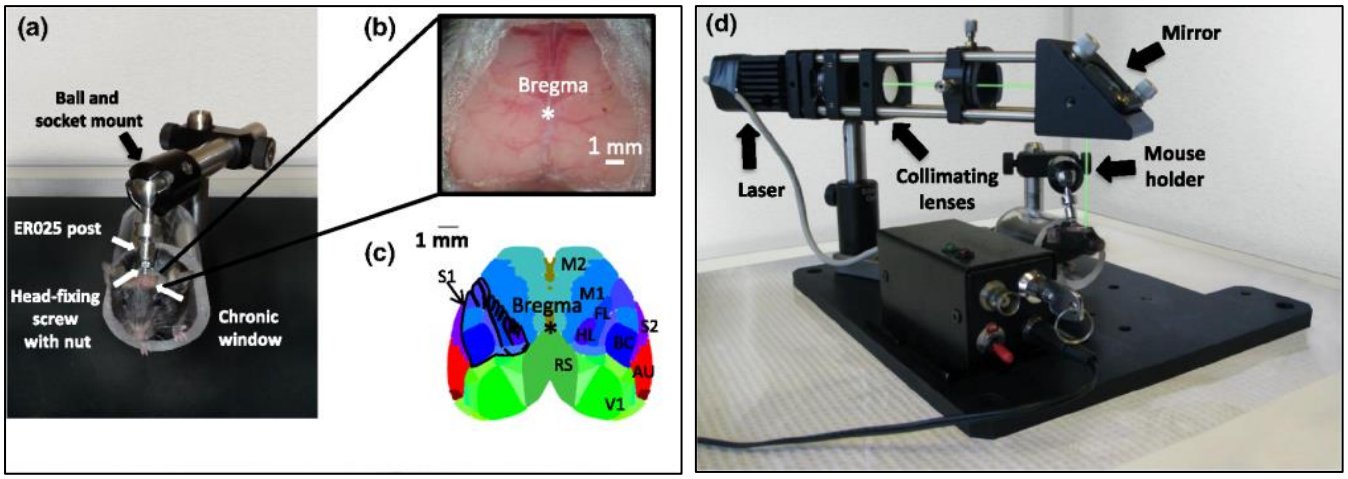
- This procedure is undertaken at least one week post window implant surgery to allow for full recovery of the mouse.
- Always wear safety goggles when using the laser.
- During stroke induction keep the room lights dimmed and noise minimized to increase the comfort of the mouse.

III. EQUIPMENT

- PPE*

Minimum PPE is gloves, gown and mask, additional PPE may be required based on facility or additional risk e.g. working with infectious animals.

- Special safety laser glasses are required when operating the laser.
- Appropriate trolley for transporting cages.
- Disinfectant*, little brush, and paper towel for cleaning equipment.
- Head fixing apparatus with Plexiglass tube (28mm diameter) for awake mice head fixation
- 532 nm custom made laser MGM-20 (Beta Electronics, Columbus, Ohio) attenuated to 11 mW at the window surface through a polarizer.



Hardware for awake head-fixation and stroke induction. (a) A set screw was surgically implanted behind the cranial window to stably head fix the mouse. (b) Blow up of the chronic window preparation where surface vessels are visible through the

intact skull, scale bar of 1 mm. (c) Mouse brain atlas image showing cortical areas that may be targeted with this method. (d) Mice are persuaded into a chamber mounted on the system for the stroke induction, and head-fixed while awake (Images sourced from Balbi et al, (2017).

- Insulin syringes, 0.5ml, 50 units, 28G fixed needle
- Accurate animal weight scale

IV. HABITUATION

- Mice are head fixed, to prevent movement of the skull, using an apparatus consisting of a platform where a clear Plexiglass tube (28mm diameter) is attached to provide a chamber for the mouse.
- Mice are introduced to the apparatus and head-fixed daily for a few minutes until they can be gently persuaded into the tube. Performed daily for a week prior to induction of stroke.
- Mice will voluntarily enter and explore the tube as they prefer small places. Nesting material is also added initially so that they recognise the smell and freely enter.

Once habituated, mice show minimal signs of struggling and can show normal behaviours, such as whisking and grooming.

V. PROCEDURE

1. Rose Bengal (RB – a photosensitive dye solution) is freshly prepared (0.01 g of RB in 1 ml of saline solution is vortexed and sonicated, then filtered using a 0.2 µm sterile filter).
2. Handling of rodents as per: LAB_006 Handling and Restraint in Mice and Neonates
3. The mouse is head-fixed, and the area of interest is then chosen using bregma as a landmark as shown in diagram above.
4. The laser is briefly turned on (up to 30 sec max) and targeted to the correct area within the cranial window using stereotactic coordinates. The laser is preset to the necessary settings and requires no other adjustment during experiments.
5. Once correctly targeted, the laser is turned off, and all the clamps are tightened to prevent movement.
6. Using an insulin syringe (0.5ml, 50 units, 28G fixed needle), mice are injected intraperitoneally (0.1 cc per 10 g of body weight) with Rose Bengal by gently holding the tail of the mouse and lifting it up to get clear view of the abdomen. The SOP LAB_028 Injections - Intra-peritoneal (IP) in Mice, Rats and Neonates should be followed. Any deviations from this SOP should be stated in the ethics application.
7. After injection allow 2 min for the dye to reach the brain.
8. Laser illumination is then turned on for 13 min at the targeted area to induce a focal ischemia.
9. Monitor the mouse during the whole head fixation duration to look for any potential complications including signs of the mouse struggling or distress.
10. After the 13 min stroke induction, make sure the laser is turned off and safe, and return the mouse back to its home cage. The mouse should be monitored for 1 hour following the procedure.
11. This procedure introduces a focal lesion that does not induce large deficits in function. The mice are monitored by the research team daily using the generic score sheet for a week post procedure.

VI. REFERENCES

1. Matilde Balbi, Matthieu P. Vanni, Gergely Silasi, Yuki Sekino, Luis Bolanos, Jeffrey M. LeDue, Timothy H. Murphy, "Targeted ischemic stroke induction and mesoscopic imaging assessment of blood flow and ischemic depolarization in awake mice," Neurophoton. 4(3) 035001 (14 July 2017) https://doi.org/10.1117/1.NPh.4.3.035001