Stroke Induction

Fiber optic light guide (tip: .89mm (20 GA) inserted into 15V fiber optic lamp. 15-20 W output verified using light meter.

Light guide is mounted onto stereotaxic frame.

Mice are anesthetized with1.5% isoflurane and mounted in stereotaxic frame. Core temperature is maintained at 37°C with feedback controlled heating pad.

Make midline incision exposing skull. Drill used to thin skull section approximately 0.5 mm medial and 0.5 mm lateral to bregma. (Skip this step if mice have cranial window)

5 ml of 10 mg/ml rose bengal is made with 5 ml of saline and 0.05 g of rose bengal.
Mice are then intraperitoneally injected with 150 μm rose bengal. Wait five minutes before stroke induction.

Light guide is then lowered to touch the window/ thin skull section for 15-20 min (~15 min if cranial window).

Incision is sutured and mice are returned to individual housing.

After 1-2 wk mice are anesthetized with IP injected ketamine (100mg/kg) and xylazine (10mg/kg) and then perfused with ice-cold phosphate buffered saline followed by 4% paraformaldehyde.

Full brains are then cryoprotected with two subsequent overnight incubations in 15% and 30% sucrose in PBS at 4°C.

Brains are then sectioned on a sliding microtome (40 μm thick). Sections are washed in PBS and blocked with 5% normal goat serum and 0.3% TritonX in PBS at room temperature for 2 hr.

Sections are incubated in primary antibody (1:6000 chicken anti-GFP, 1:500 rat anti-CD13) diluted in 5% normal goat serum and 0.3 TritonX in PBS overnight at 4°C.

Sections are later incubated in secondary antibodies for 2hr (1:1000 488-labeled/GFP goat anti-chicken, 1:1000 Cy5-labeled goat anti-rat)

Sections are then mounted on slides.

Final Volume (ml)	**Weight of Rose Bengal (g)**
2	.02
5	.05
10	.1