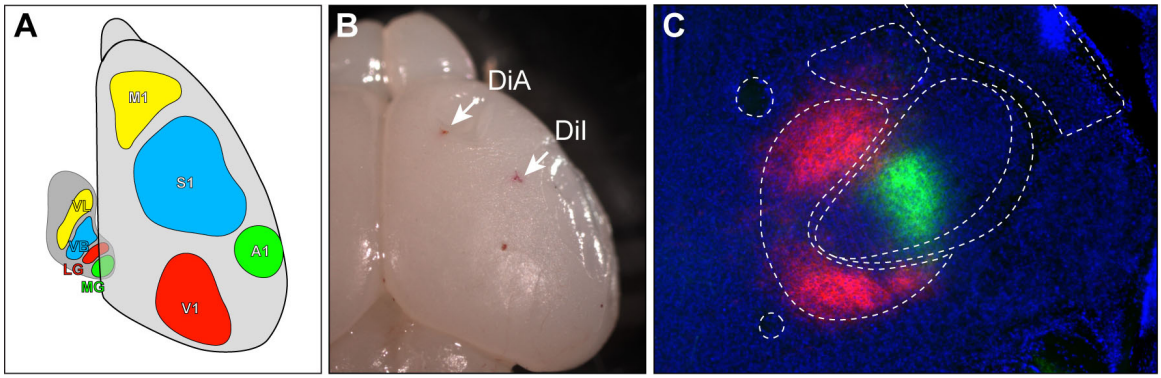
# DiI/DiA axonal tracing

1. Brains are dissected in PBS, and post-fixed with immersion in 4% PFA at least overnight. (Labeling can be done after one week in PFA)
2. Determine the dye placement location under a dissecting scope. You can first take a digital image of the brain and superimpose a pre-constructed grid on the image to determine the location.
3. Make a small hole in the pia at the insertion site using a 36-gauge syringe needle.
4. Use insert pins (No. 26007-02 and No 26018-17 or 26016-12, FST) to insert a single DiI or DiA crystal (D3911 and D3883, Invitrogen) into the brain tissue. Make sure to use the same pine for a particular dye. It is also important not to penetrate the crystal into the underlying white matter.
5. Image the brain to record dye placement.
6. Keep brain in 4% PFA at 37˚C. The time of incubation depends on the stage of the brain (see following table).
7. Once diffusion is complete, the brains are sectioned with a vibratome at 100 $\mu$m thickness.
8. Sections are mounted with 30% sucrose. (Don't use commercial mounting media, which often contain glycerol). Make sure no detergent is present during sectioning and mounting.
9. Take picture as soon as possible.

Table I (based on Gurung & Fritzsch, *J Comp Neurol,* 2004)

| Age | E13.5 | E14.5 | E16.5 | E18.5 | P0 | >P2 |
|---|---|---|---|---|---|---|
| Days | 8 | 10 | 10-13 | 14-15 | 18 | 4-6 weeks |




A. Schematic representation of topographic connections between specific cortical areas with thalamic nuclei. B. Insertions of DiA and DiI crystals. C. Retrograde labeling of thalamic neurons.