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 µm 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)

<table>
<thead>
<tr>
<th>Age</th>
<th>E13.5</th>
<th>E14.5</th>
<th>E16.5</th>
<th>E18.5</th>
<th>P0</th>
<th>&gt;P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>8</td>
<td>10</td>
<td>10-13</td>
<td>14-15</td>
<td>18</td>
<td>4-6 weeks</td>
</tr>
</tbody>
</table>

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.