Dye Tracing Protocols

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KM Lab Genetics TCD

Post mortem dye tracing (DiI, DiA)

Use crystalline DiI (Invitrogen DiIC_{18}(3) D-3911) or DiA (Sigma D-4167) for double labeling (additional dyes (e.g. DiO) can be used for more complicated tracing experiments).

After perfusion and post-fixing, dissect brains to reveal tracing site.
For dorsal thalamic tracings, divide brains along midline and remove the hidbrain. Using a sharp microsurgical knife (WPI M411880) dissect away part of the diencephalon to reveal the dorsal thalamus being careful not to cut into the telencephalon.
Insert a small crystal of dye (50-100μm) into the thalamic nucleus of interest using a fine probe (WPI #500133). For cortical or ventral telencephalon tracings simply insert crystal of dye into region of interest using fine probe taking care to insert the crystal just sufficiently deep to trace from region of interest. Incubate tissue in PBS containing 1% PFA for 2-10 weeks at RT.

Note: the diffusion of dye is temperature dependent –
@ 4ºC diffusion is extremely slow but is mostly confined to diffusion along axons, with very little diffusion to neighbouring cells.
@ 10-20ºC diffusion is faster but there is some diffusion at the site of dye placement.
@ 37ºC diffusion is very fast (P0 brain TCA pathway takes 8-10 days) but there is significant diffusion at the site of dye placement.

Dyes can also be used to trace axons in *in vitro* slice culture assays and explant assays as an alternative to Immunohistochemistry.

Live dye labeling of axons (DiI)

DiI works best for tracing cortical axons in live animals (mice from P4-P8, though older may also be possible). Anaesthetize animals by cooling on covered ice pack. Carefully cut scalp to reveal skull and using a 26G needle make small hole over cortical region of interest. Inject 1-2μl of DiI solution (10mg/ml dissolved in 100% ethanol). Cover site of injection with scalp and seal with tissue glue (e.g. dermabond). Warm under a heat lamp and return pup to the mother for 24-48 hours before sacrificing. Perfuse animals with PBS then decapitate, remove top of skull and fix for 24 hours by immersion in 4% PFA (perfusion with PFA will distribute tiny DiI particles throughout the vasculature in the brain making visualization of axons difficult).

Brain tissue is sectioned on a vibratome @ 30-100μm and <u>sections must not be allowed to dry out</u>. DAPI should be used to stain cell nuclei and slides coverslipped with Aquapolymount or Vectashield. Store @ 4ºC although DiI will continue to diffuse so sections should be photographed as soon as possible (ideally the same day!!!).