Cytochrome Oxidase Reaction for Flattened Cortex (modified from DE Feldman)

Perfusion (desired but optional)

~ 10 min: Wash with 100mL phosphate buffer (PBS; 0.1 M) + 0.5mL heparin + 1mL lidocaine (filter w/ qualitative fluted paper before use).

~ 15 min: Fix with 4% paraformaldehyde in PB (2.9 % PFA) with 2% sucrose

No perfusion - typical after in vivo recording (decapitate and keep craniotomy open.

3 - 5 days: Immerse head in 2.9 % paraformaldehyde in 0.1 M PBS with 10 % sucrose (4^{o}C)

Tissue Preparation

~ 10 min: Remove brain, isolate cortex, trim down to ~ 1 cm square, and flatten between saran-wrap covered slides using 2-mm or 3-mm spacers. Hold with rubber bands.

During dissection, make slit at rostro-caudal slit at rostromedial edge of tissue square, to preserve orientation information

4 hrs: Postfix in 2.9 % PFA with 20 % sucrose

Overnight: Sink cortex, still between slides, in 2.9 % PFA with 20 % sucrose

Reaction

~ 1 hour: Section on freezing microtome at 50 to 100 µm thickness; make alignment marks before cutting. Patrick uses 10 µm for his flattened cortex sections - it's easier and contrast is as good or better than 50 µm. Collect sections in PBS in 48-well plates.

Sections can be stored overnight in PBS, refrigerated, before reacting.

DAB reactions require oxidant-free, i.e., absolutely bleach-and soap-free, glassware and wells. Any bleach or soap residue will cause non-specific DAB precipitation and uniformly dark sections. Thus rinse all glass and plasticware carefully before use and do not use paper towers to dry. Never use bleach to decontaminate the 48-well plates or paintbrushes. Use dedicated glass and plasticware for this reaction. **

3 x 10 min: Wash sections in PBS

4 - 6 hr: React with DAB reaction mixture in the dark at 40-41^{o} C in a 48-well plate, covered in two double-layers of aluminum foil to prevent water bath fluid from seeping into the plate. The foil-covered plate should sit of float in the bath with a low water level.

Monitor reaction and stop when barrels are dark and well differentiated.

Allow to run overnight if necessary.

3 x 3 min: Rinse with PB to end the reaction. Can keep overnight at 4^{o} C if required.

~ 30 min: Mount sections on slides

1 - 2 hrs Air dry slides

Dehydrate in serial ethanol precents, e.g., 30s in 95%, 30s in 99%, 60s in 100%.
Defat in xylenes; 30s in Xylene-I, 2 min in Xylene-2
Coverslip with Permount

Solutions

Perfusion Wash: 100mL of M PB (pH 7.4) + 0.5mL heparin (1000 U/mL) + 1mL lidocaine. Filter before use.

Perfusion Fix: 2.9% paraformaldehyde in PBS + 2% sucrose. Filter before use.

DAB reaction mix: 37.5 mL 0.1M PB + 1.5g sucrose + 33mg DAB tetrahydrochloride (Sigma D5637-5G) + 15mg cytochrome C (Sigma C-2506). Filter before use.