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Embedding One Brain For Serial Section Imaging

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Rob Campbell[1]

[1]Sainsbury Wellcome Centre, UCL

Rob Campbell
Sainsbury Wellcome Centre, UCL

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We use this protocol and it's working

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Abstract

Describes how to embed one perfused mouse brain for serial section microscopy using the SWC's embedding molds.

Guidelines

It helps to have a stock of the 4% agar. Do not boil this stock: aliquot the required amount and work with that.

Materials

- ~50 ml beaker
- Embedding mold with steel wires (guitar strings work) to support brain
- Forceps

Safety warnings

This protocol uses no dangerous substances.

Ethics statement

This protocol does not describe perfusion or tissue collection. Please follow all relevant government and ethical guidelines when sourcing tissue for this protocol.

Before start

A major advantage of serial sectioning is that it generates a seamless 3D data set that can be registered to a standard atlas. For this whole process to work well the sample should be well perfused (no blood) and be undamaged. Badly perfused samples can cut poorly, producing artefacts. Damaged brains tend to register badly to the atlas.

- Post-fix overnight in 4% PFA at 4 degrees C. This is more important for rat brains: these may need two days.
- Store in regular PBS or 50 mM PB at 4 degrees C for at least 12 hours before imaging. There is usually PB down in the AMF prep area in 2L bottles. Avoid going straight from PFA to 50 mM PB then imaging the same day: these brains tend to swell whilst they are being imaged. (Sucrose is not involved: we aren't using a cryostat.)
- Longer term storage (months) is not encouraged because brains deform over time. If necessary, however, use 0.01% azide **and label as such**. Do not use higher azide concentration: that stuff is really dangerous. **Thoroughly wash all samples stored in azide before bringing them down for sectioning**.

Preparing for agar embedding

1 Pour fluid out of the sample tube and leave brain on a piece of absorbent paper, ready for use. You will need serial sectioning agar stock solution before proceeding.

Protocol

NAME

BrainSaw agar stock solution

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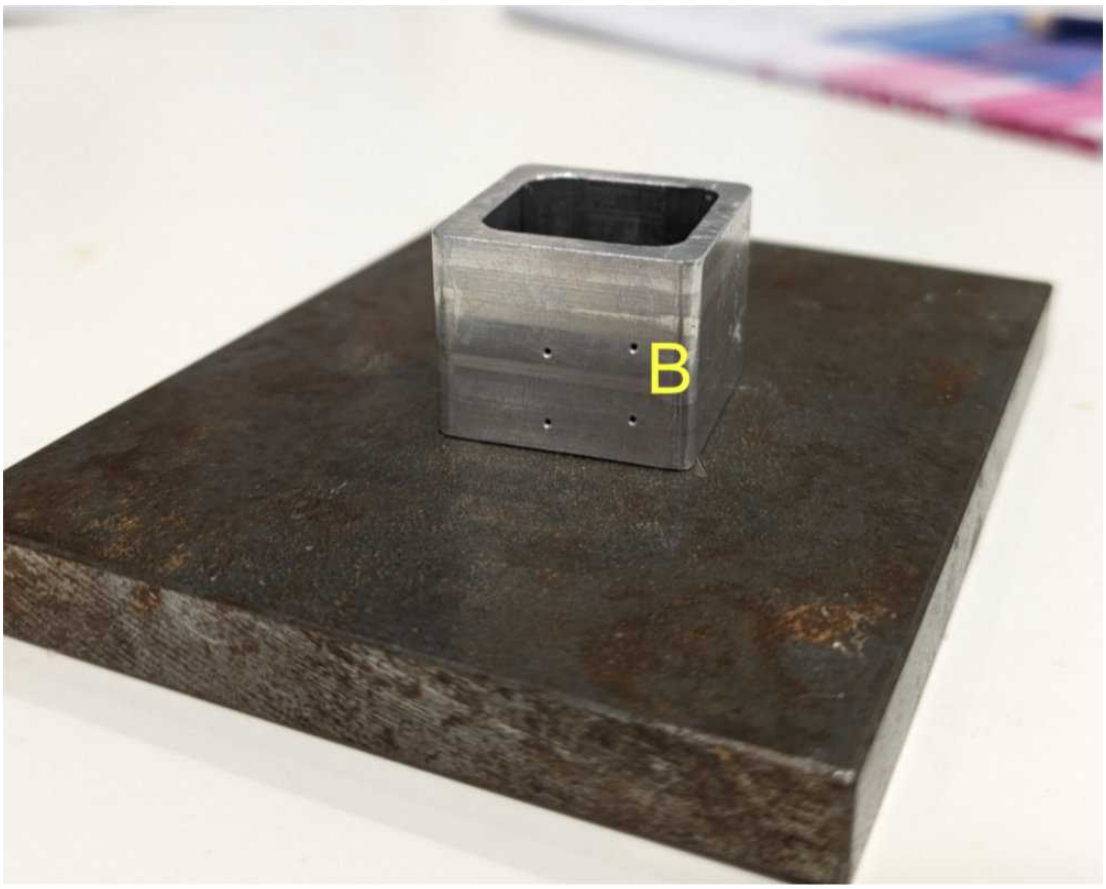
Rob Campbell

PREVIEW

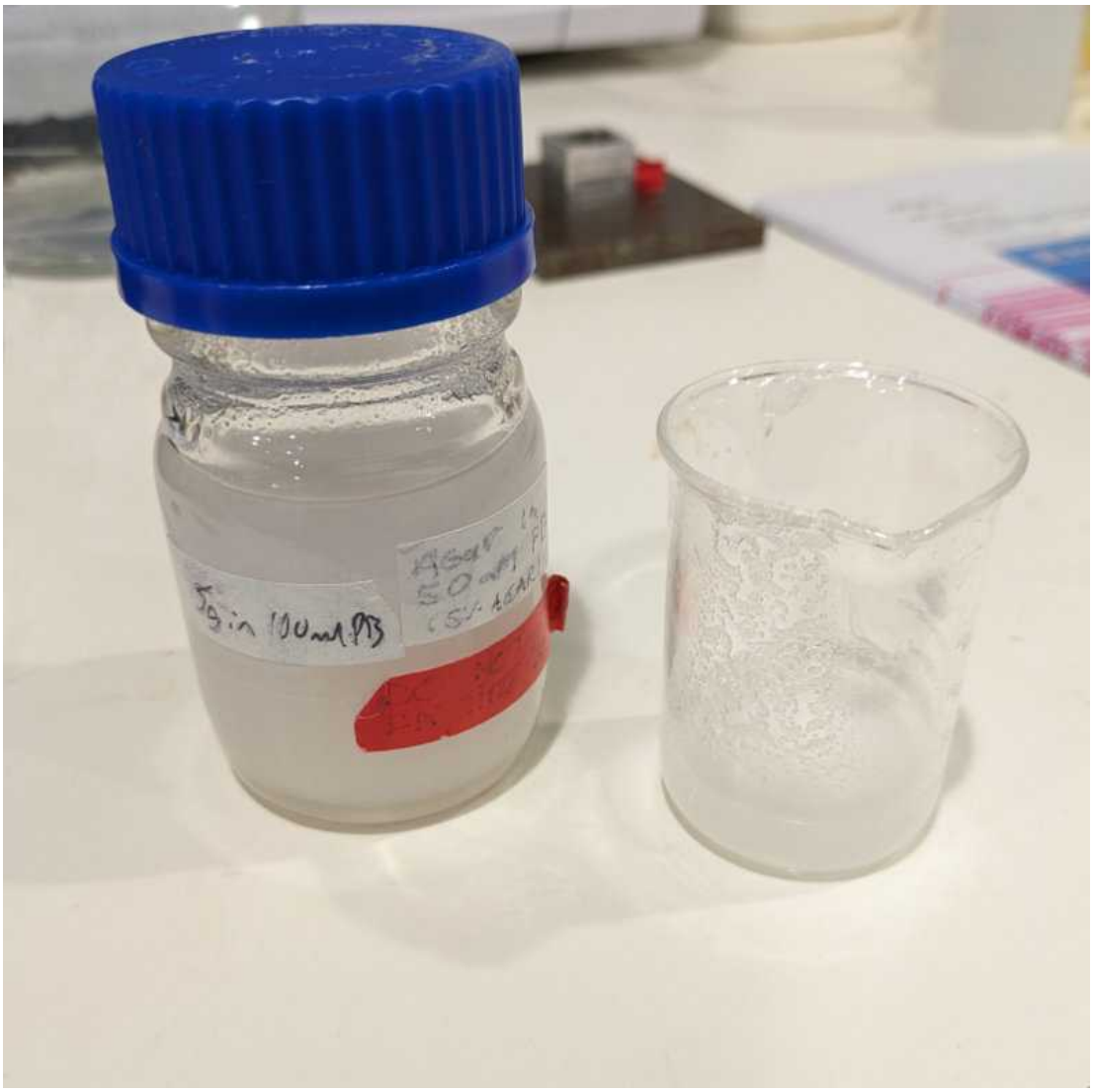
2 Place the square (single brain) metal mold on the metal plate as shown. You will use the upper row of holes. Ignore the lower holes: this mold is not used for two brains. The olfactory bulbs will rested on the wire which passes through the indicated hole. This will ensure the ventral brain surface is roughly parallel with the table.



3 Insert two wires (tape colour means nothing) through the upper holes and use these to support the brain.

Pouring the initial agar

4 Briefly shake the agar suspension then pour just over 10 mL of it into a small beaker.



5 Set microwave to low or medium power and heat carefully. Watch carefully so it does not over-boil. It is sufficient if the agar has only just melted. It does not have to boil thoroughly. Don't worry about air bubbles.

The agar is ready to use once it is about about 50 °C to 60 °C You can either use a thermometer or simply judge it according to when the underside of the beaker is no longer painful when pressed against your wrist.

6 Hold the brain down gently with forceps using your dominant hand then pour the agar until the mold is filled. Go quickly: if the agar happens to already be a bit on the cooler side then it it will set as you pour onto the metal. Tweak brain position if needed to ensure it is square in the mold.

Trim agar block and mount on slide

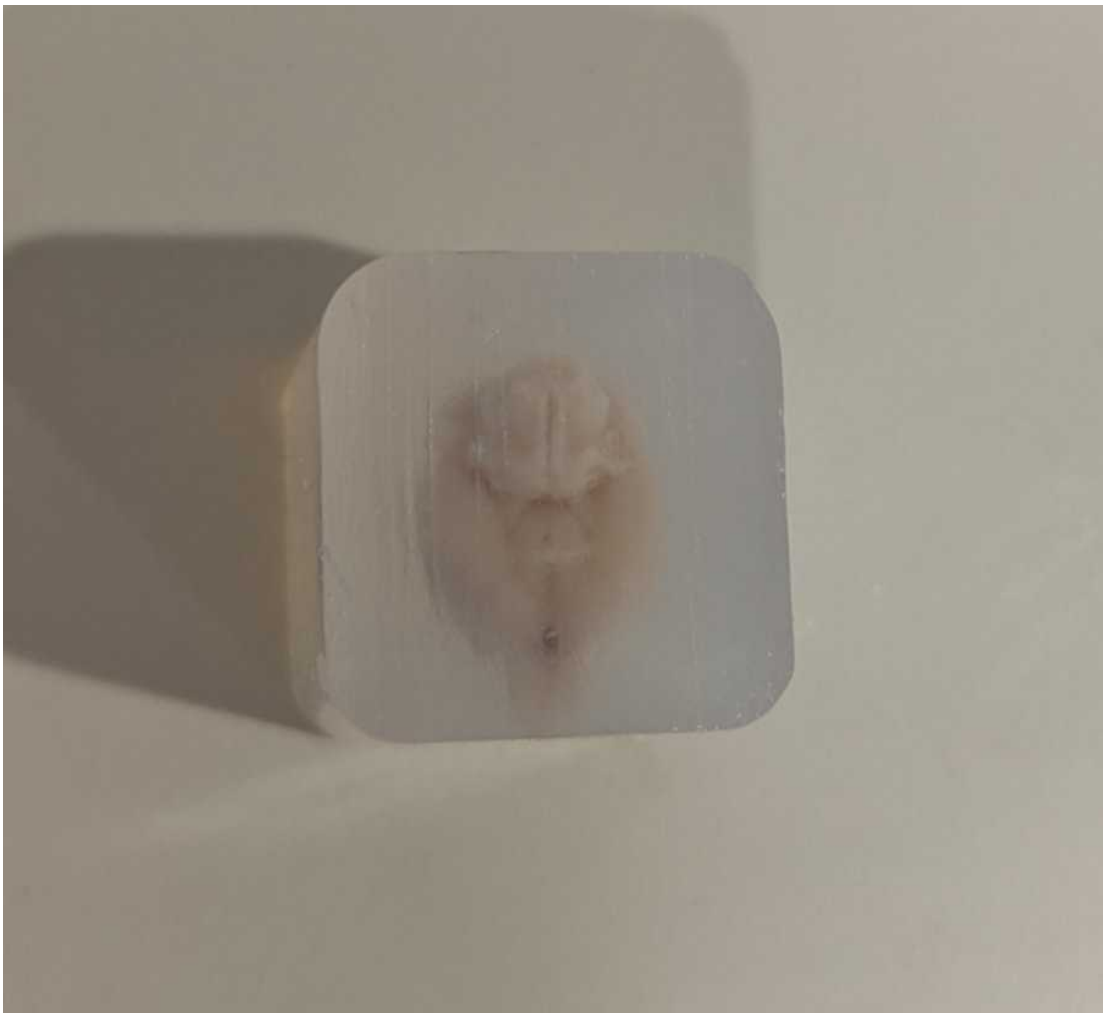
5m

7 Wait for agar to cool. This is faster in the fridge.

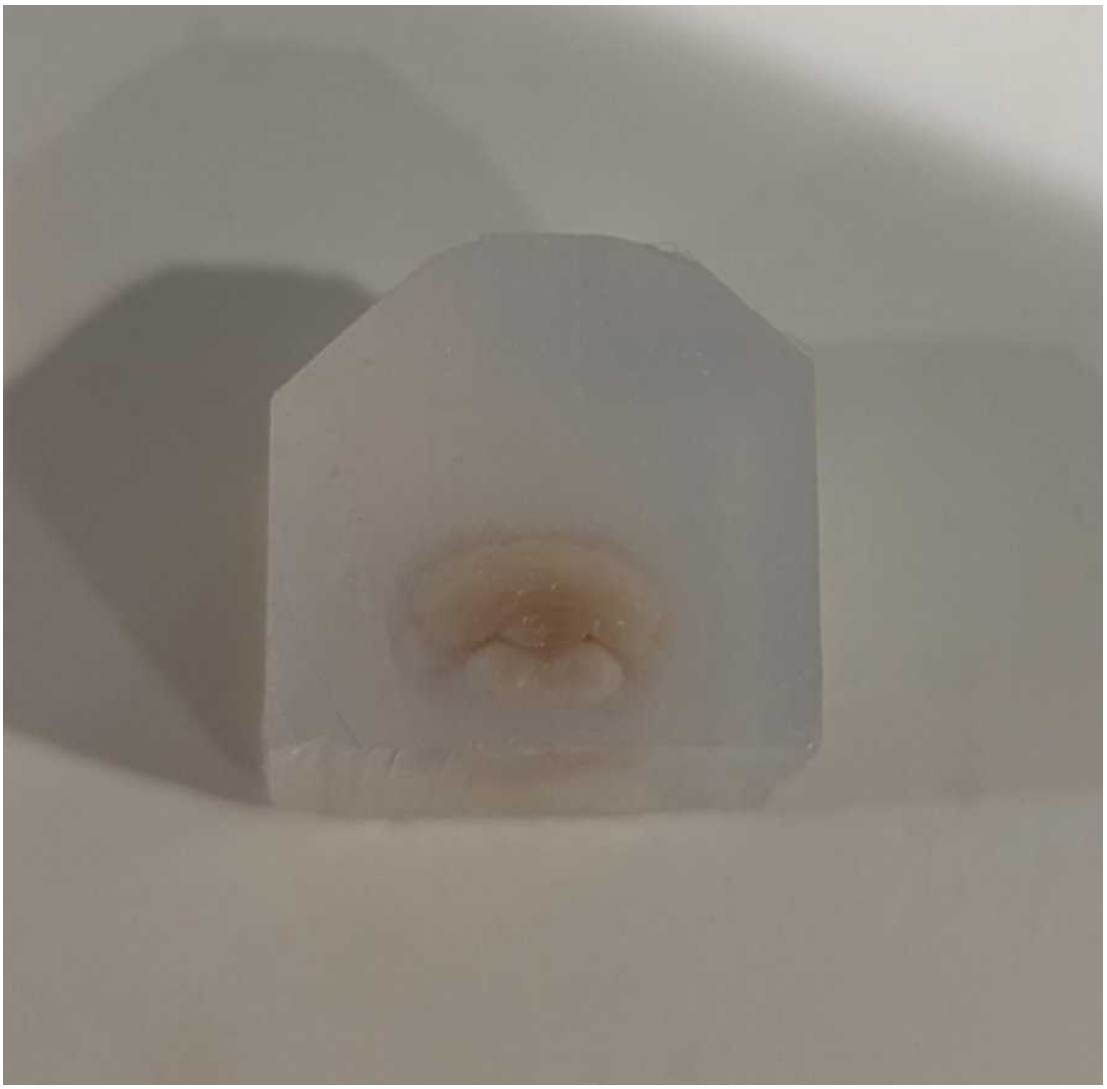
5m

8 If agar is projecting out of the top of the mold, you can trim it flat with a rase. Then pop the agar out of mold.

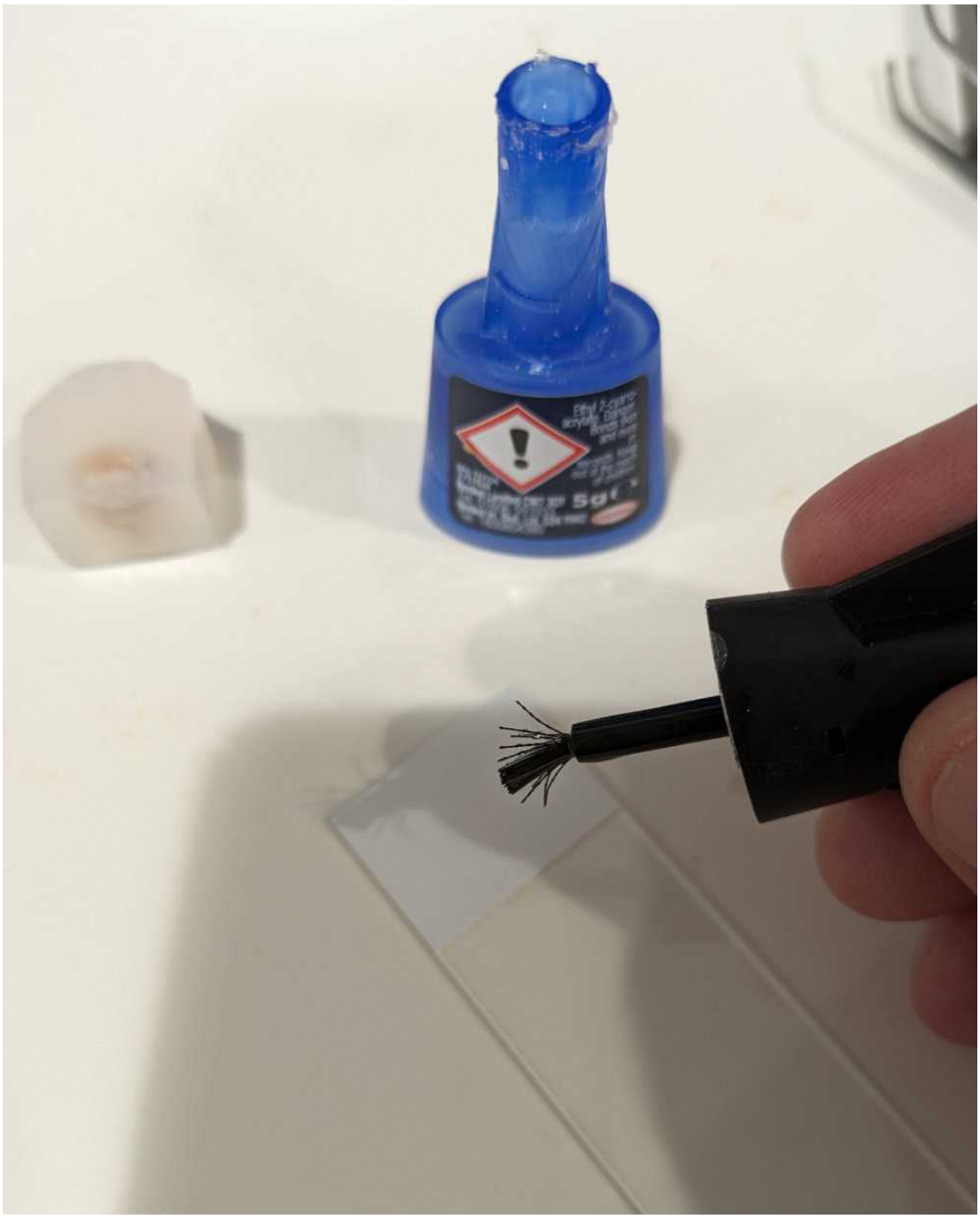
9 The system will cut from posterior to anterior. There will probably be a good deal of agar "above" the cerebellum, as shown below. It will be annoying and time consuming to trim this on the vibratome, so trim it now with a razor.



10 The blade will run from ventral to dorsal side. Cut a curve into the "dorsal" side to minimise the chance of slices remaining attached to the block after the blade has passed. **Do not trim the agar tightly around the block**: this increases the chance of the block tipping over during imaging and a tightly trimmed block can interfere with the algorithm that identifies the tissue to image only that.



11 Apply superglue to the frosted portion of the slide. Aim for a spot roughly 1 cm in diameter.



12 Place agar block on to the glue and ensure the brain is square with respect to the slide. i.e. not rotated. It will take a minute or so for the glue to set.