Preparation of rat organotypic hippocampal slice cultures using the membrane-interface method

Timothy W. Church and Matthew G. Gold

Department of Neuroscience, Pharmacology & Physiology, University College London, Gower Street, LONDON, WC1E 6BT, UK.

Correspondence should be addressed to M.G.G. (m.gold@ucl.ac.uk)

Abstract

Cultured hippocampal slices from rodents, in which the architecture and functional properties of the hippocampal network are largely preserved, have proved to be a powerful substrate for studying healthy and pathological neuronal mechanisms. Here, we delineate the membrane-interface method for maintaining organotypic slices in culture for several weeks. The protocol includes procedures for dissecting hippocampus from rat brain, and collecting slices using a vibratome. This method provides the experimenter with easy access to both the brain tissue and culture medium, which facilitates genetic and pharmacological manipulations and enables experiments that incorporate imaging and electrophysiology. The method is generally applicable to rats of different ages, and to different brain regions, and can be modified for culture of slices from other species including mice.

Keywords: organotypic, hippocampus, brain slice, whole-brain cultures

Running head: Organotypic slice culturing

1. Introduction

The first studies incorporating organotypic slice cultures utilised the roller tube technique [1, 2], in which brain slices attached to a cover slip are placed in a roller tube. This approach has been largely superseded by the membrane-interface method [3], which is the focus of this chapter. Since its introduction in 1991 by Stoppini, Buchs, and Muller, this simple and inexpensive method has been cited in over 4,000 publications. The interface method involves placing brain tissue slices on a semi-permeable membrane insert, which is placed in a well containing culture medium (**Fig. 1**). Nutrients in the slice culture medium reach the brain tissue via capillary action, and – since the tissue is only covered by a thin film of solution – sufficient oxygenation occurs with incubation in a humidified atmosphere.

Organotypic slices cultured using this approach were first used to study neuronal development, synaptic plasticity and synaptogenesis [3–6]. These original studies revealed that the maturation of synaptic connections during *ex vivo* culturing is similar to *in vivo* maturation, highlighting the value of organotypic slices as a substrate for studies of development. Importantly, the interface method provides direct access to slices and their extracellular environment. As such, the technique is highly compatible with imaging and electrophysiology experiments and enables direct application of pharmacological compounds to either the slice or culture medium. For example, organotypic cultured slices have been used in high-content drug screening studies [7, 8]. Easy access to the brain tissue also provides the opportunity for genetic manipulations [9, 10], e.g., to drive protein overexpression [11, 12] or knockdown [11]. Genetic manipulations can be induced by biolistic transfections [13], viral infection [11] or single-cell electroporation [14]. The use of organotypic brain slice cultures from animal models of neurodegenerative disease including Alzheimer's disease [15–17] is increasing. In addition, the culture medium may be monitored over time for the presence of diagnostic molecules. For example, levels of lactate dehydrogenase and inflammatory cytokines can be analysed evaluate cell damage, neuroprotective mechanisms, and ischemic events [18, 19].

It is worth considering the value and limitations of organotypic slice culturing compared to other methods for studying neurons. Primary cell cultures provide the most homogenous preparations that facilitate imaging of single neurons and are invaluable for functional analysis of, e.g., ion channels and metabotropic receptors. However, they have limited utility for investigating neuronal circuitry and plasticity mechanisms. Studying neurons in living animals is technically very demanding, although there have been many recent developments in imaging [20, 21], electrical recordings [22], and genetic manipulations [23] supporting research at this end of the complexity spectrum. Experiments with brain slices have the advantage that the number of animals required to test a hypothesis can be reduced in many cases since 10-15 hippocampal slices at a thickness of 300 μM can be collected per P17 male Sprague Dawley rat [17].

Acute brain slices are sufficient for many experiments, but viability is limited to about 24 hours in the absence of culturing. There is ongoing debate concerning the suitability of organotypic cultures for studying the properties of neuronal networks. Clearly, slicing severs many axons and dendrites [24] but certain pathways may be well preserved following slicing [25]. The architecture of the hippocampus is particularly well suited to experimentation in slices. For example, intact Schaffer collaterals may be obtained by slicing in any of a number of different planes [25], including the horizontal plane as outlined in this chapter. Organotypic culturing enables some recovery of axonal projections, the dendritic arbour, and synaptic connections over time following slicing [26, 27], providing a potential advantage in comparison to acute slices. This perspective is supported by a comparative study, which showed that spontaneous spike firing rates and excitatory synaptic inputs are more similar to the *in vivo* hippocampal network in organotypic slices than acute slices [28]. However, this recovery in synaptic connections reaches a tipping point after about two weeks of organotypic culturing with rodent hippocampal slices, with excitability reaching levels that trigger epileptic events that preclude electrophysiology experimentation [5, 29]. Overall, organotypic culturing has clear value, and in many cases synergises with studies at higher and lower levels of complexity.

In this chapter, we detail the materials required for the interface method of organotypic slice culture (Section **2**) before laying out preparatory steps (Subheading **3.1**), and protocols for dissection and slicing of hippocampus from rats (Subheading **3.2**), and establishing organotypic cultures (Subheading **3.3**). We also describe necessary steps during organotypic culture (Subheading **3.4**), and explain how to remove slices from culture for experimentation (Subheading **3.5**). Common pitfalls, potential modifications, and theoretical insights are highlighted in 'Notes' (Section **4**).

2. Materials

Prepare solutions using ultrapure water (e.g., by purifying deionised water with a MilliQ system to a resistivity of > 18 MΩ), and analytical grade reagents.

2.1. Dissection and slicing

1. Sprague-Dawley or Wistar rats aged postnatal day 17-19 (*see* **Note 1**).
2. Dissecting medium: 189 mM sucrose, 10 mM D-glucose, 26 mM $NaHCO_3$, 3 mM KCL, 5 mM $MgSO_4.7H_2O$, 1.25 mM NaH_2PO_4, 0.1 mM $CaCl_2$. Store at 4 ˚C for up to one week (*see* **Note 2**).
3. Artificial cerebral spinal fluid (aCSF): 124 mM NaCl, 3 mM KCl, 24 mM $NaHCO_3$, 1.25 mM $NaH_2PO_4.H_2O$, 1 mM $MgSO_4.7H_2O$, 10 mM D-glucose, 2 mM $CaCl_2$. Store at 4 ˚C for up to one week (*see* **Note 3**).
4. Vibrating blade microtome (*see* **Note 4**), e.g., Leica VT1200 S (Leica Microsystems).
5. Stainless-steel blade, e.g., Wilkinson Sword double-edged blade (*see* **Note 5**).
6. 70 % Ethanol.
7. Dissecting tools: large scissors, small dissecting scissors, dissecting tweezers, blunt rounded spatula, scalpel and blade.
8. Dissecting microscope, e.g., Zeiss Stemi 2000c stereo microscope.
9. Parafilm.
10. Cyanoacrylate adhesive, e.g., Loctite superglue.
11. Slice holding chamber (e.g., from AutoMate Scientific Brain Slice Keeper series). A homemade holding chamber can be fabricated from a 50 mL syringe and a fine nylon mesh (**Fig. 2**, *see* **Note 6**).
12. 95 % O_2 / 5 % CO_2 gas mixture (carbogen).

2.2. Slice culture

1. Osmometer, e.g., Vapor Pressure Osmometer M5520 (Wescor).
2. Penicillin-streptomycin (5,000 U / mL penicillin and 5,000 μg/mL streptomycin)
3. Organotypic slice culture medium (OSM): 49.4 % MEM, 12.5 % heat-inactivated horse serum (Gibco, cat. no. 26050088), 75 mM HEPES, 5 mM $NaHCO_3$, 0.625 mM $CaCl_2$, 1.25 mM $MgSO_4.7H_2O$, 1 mM L-glutamine, 32 mM D-glucose, 1 mg / L insulin from bovine pancreas (*see* **Note 7**), 0.425 mM ascorbic acid, 50 units (U) / mL penicillin, 50 μg / mL streptomycin. To prepare 200 mL OSM, add all components and bring up to ~ 150 mL before adjusting the pH to 7.28 with concentrated NaOH. Monitor the osmolarity when bringing up to the final volume using MilliQ water until a concentration of 320 mmol / kg is reached (*see* **Note 8**). Transfer OSM into a sterile tissue culture hood and sterile filter OSM through a 0.22 μm filter (e.g., Nalgene, cat. no. 568-0020). Store at 4 ˚C for up to one month (*see* **Note 9**).
4. Semi-permeable cell culture inserts (Millipore, cat. no. PICM03050).
5. Polypropylene 6-well culture plates and 10 cm diameter dishes
6. Modified Pasteur pipettes for the transfer of hippocampal slices. To fabricate wide-ended pipettes, first break off the tapering edge of a glass Pasteur pipette. Smooth the broken end with a naked flame then cover with a rubber bulb (e.g. Fischer Scientific, cat. no. 10746162) for drawing solution into the wide end of the glass pipette (diameter ~ 1 cm).

2.3. Lentiviral infection

1. 5 μL Hamilton syringe (cat. no. 549-1231).
2. Fine 32 gauge Hamilton removable needle (cat. no. HAMI7762-05).
3. Lentivirus stored in 5 μL aliquots and stored at -80 ˚C until required.
4. Pre-chilled OSM for diluting lentivirus.
5. 50 mL of 70 % Ethanol in a 50 mL Falcon tube.

3. Methods

The following procedures should be carried out in a sterile tissue culture hood using aseptic technique unless otherwise stated.

3.1 Preparatory steps prior to dissection

1. Autoclave dissecting tools.
2. At least 30 minutes before dissection, add 1 mL OSM into each well of a 6-well tissue culture plate (*see* **Note 10**). Add a Millipore cell-culture insert into each well using sterile forceps. Ensure that the OSM is in contact with the semi-permeable membrane and that no air bubbles are trapped underneath the insert. Culture dishes can be prepared up to 24 hours in advance of dissection.
3. Prepare slice washing plate: add 5 mL OSM supplemented with antibiotics into 3 wells of a 6-well plate, and transfer into in an incubator at 37 ˚C with 5 % CO_2. Washing plates can also be prepared up to 24 hours in advance. If the vibratome is cleaned diligently between slicings, it is possible to culture slices without antibiotics, but it is advisable to at least include penicillin and streptomycin in the initial washing solutions (*see* **Note 11**).
4. Spray the floor and walls of the tissue culture hood with 70 % ethanol. If the tissue culture hood has a UV light for sterilisation, turn on to further sterilise the tissue culture hood.
5. Spray dissecting area with 70 % ethanol.
6. Pour 200 mL of pre-chilled dissecting medium into a 250 mL beaker and cover with Parafilm. Place solution in -20 ˚C for approximately 15 minutes until solution forms a slurry mix of frozen and liquid solution. Once a slurry has formed, place beaker in ice and bubble dissecting medium with carbogen for at least 30 minutes.

7. Prepare 500 mL aCSF supplemented with 2 mM $CaCl_2$ and bubble with carbogen for 30 minutes.
8. Prepare dissecting area by cleaning the work surfaces and vibratome with 70 % ethanol.
9. Lay out dissecting tools and clean with 70 % ethanol.
10. Remove the double-edged slicing blade from acetone and wash with 70 % ethanol. Attach blade to vibratome mount and wash once more with 70 % ethanol.

3.2 Brain dissection and hippocampal slicing

1. Cull rats (*see* **Note 12**) in accordance with local regulations, ideally by cervical dislocation followed immediately by decapitation with large surgical scissors.
2. Spray head with 70 % ethanol to minimise contamination and keep contaminated hair fibres away from brain tissue.
3. Cut the skin using a scalpel blade in the rostal (anterior) to caudal (posterior) direction to reveal the skull.
4. Open the skull covering the cerebellum by first using the small dissecting scissors to cut from the vertebral foramen along the midline towards the sagittal suture. Next, cut at ~ 135 ˚ angles relative to the midline starting from the vertebral foramen towards each side of the skull covering the cortex (**Fig. 3a**). With fine blunted forceps, pull aside the skull to reveal the cerebellar tissue.
5. Cut the skull bilaterally along the midline from the caudal end of the brain towards the frontal lobe using small dissection scissors (**Fig. 3b**). Ensure the tips of the scissors are pressed lightly against the skull to avoid damaging the brain.
6. Using fine forceps, slightly raise one side of the skull from the brain. Use the forceps tips to detach the meninges from the underside of the skull before pulling back the skull to reveal the forebrain tissue (**Fig. 3b**). Repeat for the other side, and then remove the meninges from the exposed brain tissue with forceps.

7. Cut along the frontal plane at the division between the olfactory bulb and frontal lobes, and remove the cerebellum, using a scalpel blade (**Fig. 3c**).
8. Lift the dissected brain out with a rounded spatula and transfer into the slurry of dissection medium for ~ 1 minute.
9. The following step should be performed as quickly as possible to prevent the brain from warming up or drying out: place a layer of cyanoacrylate superglue onto the pre-chilled slicing specimen disc. For horizontal slices (*see* **Note 13**), place the brain cortex-side down onto a flat spatula and gently tap the underside of the spatula on tissue paper to drain away excess solution that might prevent the brain adhering to the specimen disc. Using the flat side of a scalpel blade, slide the brain from the spatula and onto the centre of the specimen disc in a smooth motion (**Fig. 3d**).
10. Rapidly slide the specimen disc into place in the slicing chamber, and fill the slicing chamber with the slurry of dissection medium. When filling the slicing chamber, gently pour the dissecting medium into the chamber to prevent the glue from wrapping around the brain.
11. Cut a thick ~2 mm section of brain to expose the hippocampus.
12. Set the vibratome to cut 300 μM thick slices (**Fig. 3e**, *see* **Note 14**) at a speed of 0.04 mm/sec when the blade has reached the hippocampus or region of interest. Use the modified Pasteur pipette to transfer slices to a 10 cm dish filled with aCSF bubbled with carbogen. Typically 10-15 good quality 300 μM thick horizontal slices (**Fig. 3f**, left) can be obtained from one rat brain.
13. Trim hippocampal slices by cutting from the lateral ventricle located outside of the CA3/2 stratum oriens to the lateral sides. Make a second incision along the base of the hippocampus from the lateral ventricle. Transfer trimmed hippocampal slices (**Fig. 3f**, right) to a holding chamber filled with aCSF bubbled with carbogen.
14. Inspect slices under a dissecting microscope for signs of damage caused by dissection or handling. Damaged slices should be discarded.

3.3 Establishing the organotypic culture

1. Spray outside of beaker containing slices with 70 % ethanol before transferring into tissue culture hood. Rapidly proceed to next steps if a carbogen line is not available to continue bubbling the aCSF within the hood.
2. Transfer the 6-well plate containing culture inserts along with washing plate from the incubator to the tissue culture hood.
3. Transfer slices into the first well of the washing plate using the modified Pasteur pipette and gently shake the plate in a figure-of-eight motion for 10-20 s.
4. Transfer slices into the second and then third well after gently shaking the plate in the same fashion (*see* **Note 11)**.
5. Gently transfer individual slices from the washing plate onto the membrane of the cell culture insert using the modified Pasteur pipette. Place 3-5 slices on each membrane (**Fig. 4a**) ensuring that the slices do not touch one another.
6. Remove excess media covering slices with a pipette. Take care not to touch slices once they are placed on the membrane.
7. Return the 6-well plate containing the slice inserts into the humidified incubator and culture at 37 ˚C and 5% CO_2.

3.4 Steps during organotypic culturing

1. For experiments that involve lentiviral-mediated infection, slices should be infected within 2 hours of placing in organotypic culture (*see* **Note 15**). Lentivirus may be administered using a Hamilton syringe fitted with a fine gauge needle (**Fig. 4b**, *see* **Note 16**).

2. To maintain healthy slices in all cases, replace the media every two to three days with pre-warmed OSM (*see* **Note 10**), taking care not to introduce air bubbles under the membrane. On returning the plate to the incubator, ensure that the humidification reservoir is filled to maintain a humidified atmosphere.
3. Monitor slice viability every four days under the dissecting microscope. Viable slices should appear white with well-defined Dentate gyrus, CA3 and CA1 regions. Over the first few days *in vitro*, dead cells and tissue debris are naturally cleared from the surface of the slice, resulting in slice thinning. Slices also flatten out over time in culture leading to noticeable broadening of the cell body layers. However, if cell bodies are no longer easily discernible this indicates that the slices are no longer viable and should be discarded. The appearance of black spots or webbing indicate bacterial or fungal contamination, in which case slices should be discarded immediately.
4. The Optimal timeframe to experiment with cultured hippocampal slices is between DIV6-18. Experiments become difficult due to slice thinning beyond DIV18 [30] (*see* **Note 17**).

3.5 **Removing slices from organotypic culture for further experimentation**

1. Clean a pair of thick tweezers by washing in 70 % ethanol and leave to air dry.
2. Using the sterilised tweezers, remove a Millipore insert from a well and place onto a petri-dish.
3. If the slices have been in culture for more than three days (see **Note 18**), cut through the interface membrane using a scalpel and around hippocampal slices leaving a 3-5 mm overhang of membrane around each slice. Hippocampal slices will remain attached to the interface membrane after embedding during culturing.
4. To continue to electrophysiology (see **Note 19**), transfer slices into a holding chamber filled with aCSF bubbled with carbogen using a flat-ended spatula, and incubate for one hour at room temperature prior to experimentation.

5. At high magnification, transduced neurons may be selected with the assistance of a fluorescence marker (**Fig. 4c**, see **Note 20)**, and healthy cells can be identified using infrared differential interference contrast (IR-DIC) microscopy (*see* **Note 21**).

4. Notes

1. Slices from more youthful rats (P5-P12) are more resilient to the culturing process [31, 32], especially to mechanical damage from slicing [33]. However, hippocampal morphology is better preserved when slices are taken from older rats, and culturing from P15-P19 rats is possible when slices are treated carefully [12].

2. The dissecting medium can be modified to include N-Methyl-D-glucamine (NMDG) in place of sodium [34]. The NMDG formulation can improve slice health in both acute [34] and organotypic preparations [35].

3. Prepare a 10x concentrated stock of aCSF in advance and store at 4 °C for up to one week. Working stocks of aCSF should be supplemented with $CaCl_2$ (2 mM) on the day of preparation to avoid calcium precipitation.

4. The McIlwain tissue chopper is an alternative to a vibratome. The tissue chopper is fast, easy to use, and allows for multiple hippocampi from a dozen rat brains to be cut simultaneously. However, brains are not maintained in medium during slicing, which can increase cell death, and the chopping motion compresses brain tissue, which can result in morphological changes and also cause cell death. In our experience, high quality slices are obtained using vibratomes operating at low speed (e.g., 0.04 mm/sec). Serrated surfaces can be an issue using vibratomes due to z-axis blade deflection but advanced vibratomes can compensate for z-axis deflection for smoother slicing [36].

5. Commercial blades should be cleaned in acetone before use to remove any oils coating the blades.

6. Homemade slice chambers can be fabricated from a 50 mL polypropylene syringe that is 3 cm in diameter (e.g., Henke soft-ject, VWR, cat. no. 613-1586). First, slice a 50 mL syringe (minus plunger)

into three equal open cylinders ~ 2 cm in length. Glue the three cylindrical sections together using cyanoacrylate adhesive into a triangular assembly (**Fig. 2**). After setting for 1 hour, glue a stretched nylon mesh material (e.g., tights) across one end of the triangular assembly. The slice chamber can be placed in a 200 mL beaker filled with aCSF and bubbled with carbogen to maintain healthy well-oxygenated brain slices [37, 38].

7. Prepare insulin stocks by dissolving at 1 mg/mL in 0.01 N HCl. Store at -20 ˚C in 250 μL aliquots. Insulin has been observed to improve neuronal survival rates and slice health when maintaining rat slices in culture [29].

8. Accurately titrating the pH and osmolarity (e.g., using a Vapor Pressure Osmometer M5520, Wescor) to pH 7.28 and 320 mmol / kg is critical for obtaining viable and reliable cultures. Small changes in pH and osmolarity will result in cell death.

9. Supplementing culture media with brain-derived neurotophic factor (BNDF) has been observed to produce healthy, viable slices from adult rats [39]. Alternatively, aged hippocampal slice cultures can be prepared by embedding aged brain in agarose before slicing [40].

10. Culture media in excess of 1 mL per well will rise above the level of the semi-permeable membranes and drown slices.

11. If good aseptic technique is applied, there is no need to include penicillin/streptomycin in the OSM. Penicillin is a GABA receptor antagonist at high concentration [41] so can be epileptogenic [42, 43], however, at the concentration stated in this protocol (84 μM) it should not induce epileptic activity [42]. To culture slices without antibiotics, first wash slices three times in wells filled with OSM supplemented with penicillin/streptomycin. Then wash three more times in wells filled with OSM without antibiotics before transfer onto membrane inserts sitting in medium without antibiotics.

12. The interface method is also routinely used to culture mouse brain slices [10, 31, 44].

13. Schaffer collaterals and mossy fibers are well preserved when hippocampal slices are cut in the horizontal (transverse) plane as described here. Coronal slices are obtained by fixing the brain to the specimen disc at the surface exposed after cutting between the frontal lobe and olfactory bulb (**Fig. 3c**). Sagittal slices are collected by first cutting the brain in half along the midline, and then attaching one hemisphere at a time to the specimen disc at the surface obtained from this cut [45]. Slicing along the horizontal-entorhinal cortex plane (12 ° tilt from the horizontal plane) is notable since Schaffer collaterals, mossy fibers and the perforant pathway are all preserved at this angle [46].

14. At thicknesses > 400 μM, neuronal survival is compromised by deficient diffusion of nutrients and oxygen throughout the slice.

15. Beyond two hours, a layer of astrocytes forms on the exposed upper surface of tissue once slices are placed in culture [47, 48]. This astrocyte layer acts as a barrier to lentiviral infection, which can be somewhat ameliorated by supplementing OSM with cytarabine (Ara-C) to reduce glial proliferation [49].

16. Lentivirus may be administered using the droplet method [12], or by injecting into the region of interest (e.g., CA1 layer, **Fig. 4b**). Injection may be achieved manually using a Hamilton syringe, or with an automated microinjector such as the Nanoject II Auto-Nanoliter Injector (Drummond Scientific) [50] or picospritzer (General Valve, Fairfield, NJ) [51].

17. Organotypic hippocampal slices have been reported to last for as long as 6-8 weeks when studying cell death or chronically treating slices with compounds *in vitro* [52].

18. If slices are maintained in organotypic culture for three days or less, they may be gently lifted from the inserts using the flat side of a scalpel blade after carefully pipetting 0.5 mL aCSF onto the top surface of the insert. Over time in culture, severed axons and dendrites recover and form new synapses and projections [5, 53], some of which pass through the semi-permeable membrane and anchor slices to the insert.

19. Organotypic cultured hippocampal slices are hyperexcitable. A few methods are available for reducing polysynaptic activity during electrical synaptic stimulation. An incision may be made in the CA2 region to sever Schaffer collateral connections and reduce polysynaptic activity on CA1 pyramidal neurons [54]. Alternatively, polysynaptic activity may be reduced by including the adenosine A1 receptor agonist 2-chloroadensine in the recording solution [55, 56], or increasing Ca^{2+}/Mg^{2+} concentration in the aCSF [57].

20. If a fluorescent marker (e.g., green fluorescent protein) is being used to identify transduced neurons, then neurons exhibiting fluorescent blebbing along the axons or dendrites should be avoided as this is a sign of fluorescent protein aggregation.

21. Healthy neurons visualised by IR-DIC microscopy display a visible soma with smooth edges. Cells with a swollen morphology, which display dark/high contrast edges and a visible nucleus, should be avoided.

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Figure Captions

Figure 1. Schematic of membrane-interface method. The apparatus is incubated at 37 °C, in a humidified atmosphere of 5 % CO_2. The semi-permeable membrane allows nutrients to reach the slice via capillary action without drowning the tissue in culture medium. The membrane-interface methodology affords the experimenter precise control over gene expression and culture conditions.

Figure 2. Slice chamber fabrication. A homemade slice chamber may be assembled by gluing stretched nylon tights to three cylindrical sections cut from a polypropylene syringe. Placing slices onto the porous nylon mesh enables sufficient oxygenation when slices are incubated in aCSF bubbled with carbogen.

Figure 3. Brain dissection & slicing. Throughout the figure, incision lines are denoted by dotted red lines. Panels (**a**) to (**c**) show illustrations (upper row) and photographs (lower row) for dissection to remove skull around first the (**a**) cerebellum and then the rest of the (**b**) brain prior to removal of the (**c**) cerebellum and olfactory bulbs. (**d**) Dissected rat forebrain fixed to specimen disc. (**e**) Collection of horizontal brain slices using a vibratome. (f) Horizontal 300 μM-thick brain slice (left) after vibratome slicing, and individual hippocampus (right) dissected from the larger slice.

Figure 4. During and after organotypic culture (**a**) Bird's eye view of hippocampal slices inside Millipore semi-permeable membrane insert. (**b**) Lentiviral injection using a fine gauge needle into the CA1 pyramidal layer. Subfields CA2, CA3 and the dentate gyrus (DG) are also labelled. (**c**) Example infrared (left) and GFP fluorescence (right) images of CA1 pyramidal neurons in hippocampal slices infected with lentivirus expressing GFP. (**D**) Recordings of spontaneous excitatory post-synaptic currents recorded from the GFP-labelled neuron shown in panel (**c**).

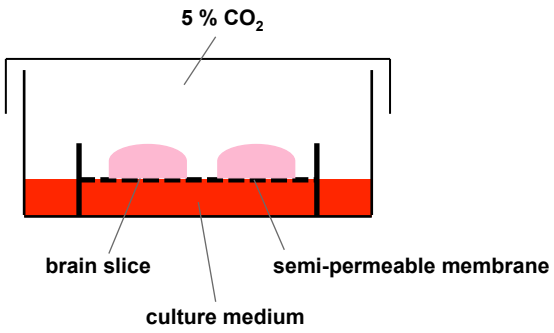

Figure 1

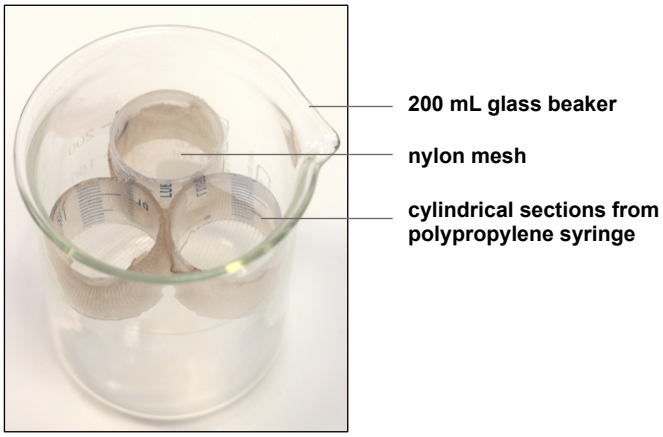
200 mL glass beaker
nylon mesh
cylindrical sections from polypropylene syringe

Figure 2

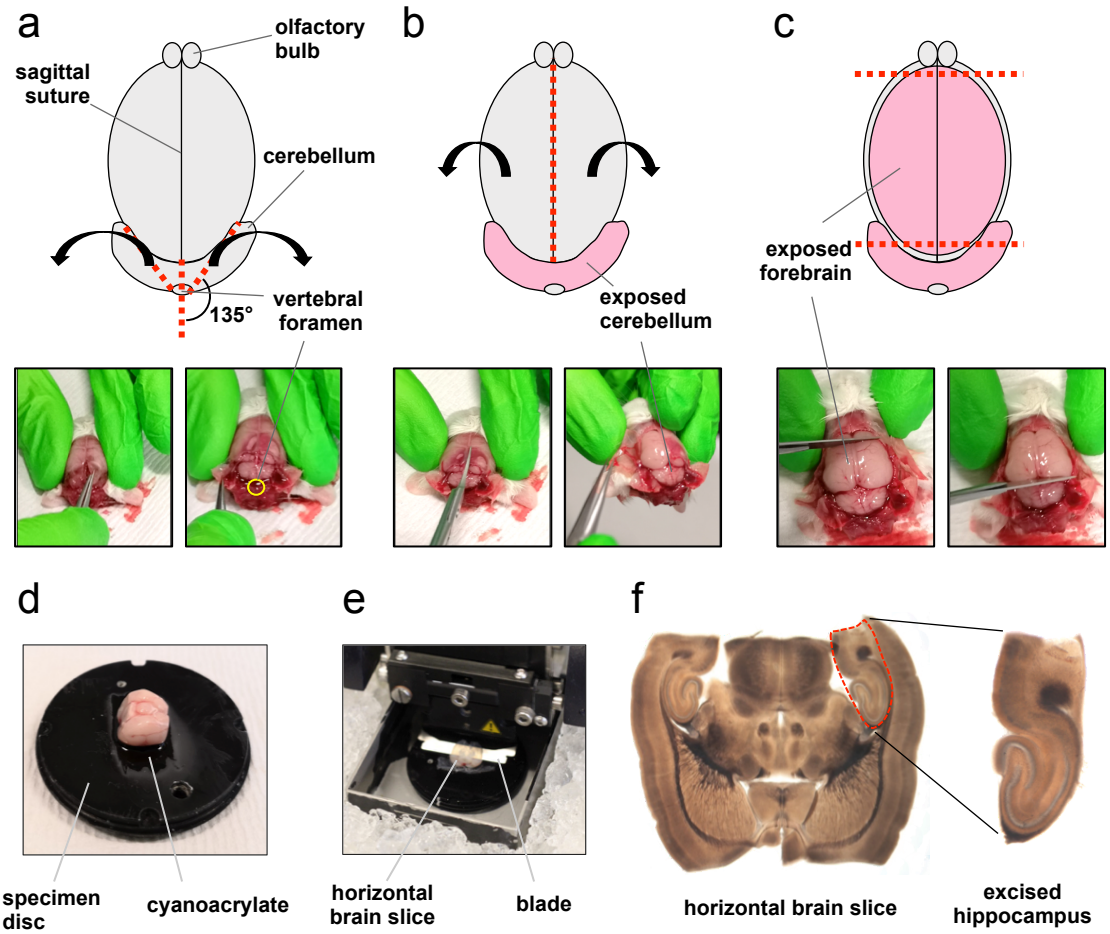
a
olfactory bulb
sagittal suture
cerebellum
vertebral foramen
135°
b
exposed cerebellum
c
exposed forebrain
d
specimen disc
cyanoacrylate
e
horizontal brain slice
blade
f
horizontal brain slice
excised hippocampus

Figure 3

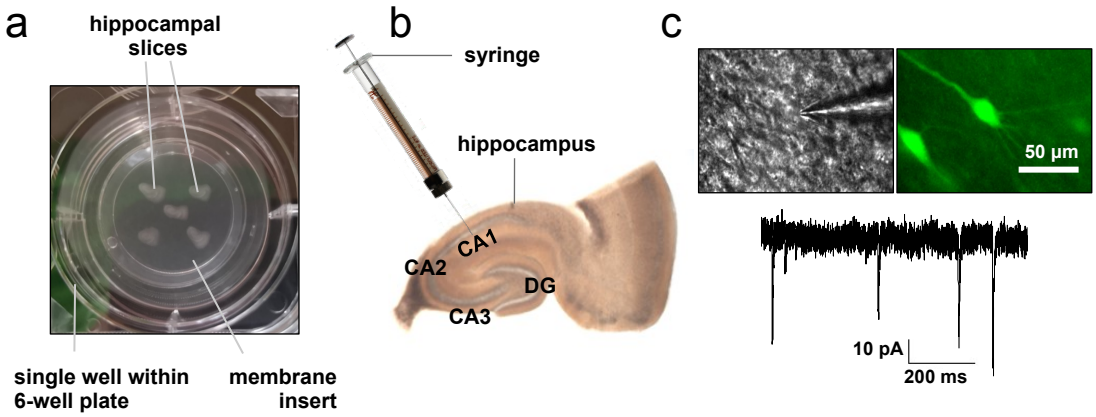
a
hippocampal slices
single well within 6-well plate
membrane insert
b
syringe
hippocampus
CA1
CA2
DG
CA3
c
50 µm
10 pA
200 ms

Figure 4