Mouse neuron dissection

(Huang et al., J Neurosci 2016; Huang et al., J Exp Med 2017; Stupack et al., J Neurosci 2020)

Note:
Mouse embryos are removed from timed-pregnant mothers typically at ~E18; neurons can be cultured from younger embryos (E15 onwards), but dissection of hippocampal neurons is optimal at E18 where the granular cell layers within the hippocampus are more visible. Surgical tools and dissection area should be sterilized before and during dissection; straight #5 forceps are primarily used during dissection.

Removing embryos:
1) Pregnant mothers are anesthetized under isofluorane, sacrificed by cervical dislocation
2) Sterilize the underside of the mother, open with scissors to expose the abdominal wall and pull the uterus with sterile forceps and cut away the uterus. Place in a 50ml tube with sterile PBS.
3) Using fine forceps, remove the embryos from the uterine sac in a 10cm dish, wash embryos with PBS and place in sterile PBS on ice
4) Remove the embryo heads with forceps or scissors and place individually in 1xPBS on ice

Dissecting the brain, isolating and plating cortical neurons:
1) Place the head in sterile HBSS in a dissection vessel (e.g. 0.5ml in a 60mm dish)
2) Remove the top of the skull using two fine forceps and carefully remove the brain
3) Dissect brain, under a stereomicroscope and lighted stage,
4) Remove the olfactory bulb and cerebellum (if not needed)
5) Remove the meninges using a pair of sterile, fine forceps
6) Carefully dissect out the hippocampus (a darker, C-shaped formation; more below), and place hippocampal and cortical tissues in separate 6-well plates containing 0.3mls HBSS (on ice)
7) Add 1ml dissociation solution: 200uls 0.25% Trypsin-EDTA + 200uls DNAseI (at 2000u/ml) in 600uls HBSS. Cut tissue with small scissors (Vannas-Tübingen spring scissors). Incubate at 37degC for 30 mins.; agitate 4 – 5 times during incubation to help dissociation.
8) Transfer the tissue/solution to 15ml conical tubes, add 3mls 1x DMEM (complete) with FBS, spin 2000 rpm for 1 min.
9) Remove supernatant, add 3mls trituration solution (1x DMEM, serum-free) and 200uls DNAseI.
10) Triturate with a 10ml serological pipette 15 times, then triturate with a fire-polished glass pipette 10 times.
11) Spin 1500rpm 3 min.
12) Remove supernatant, add 1ml each 50% 1xDMEM/Neurobasal complete mix. Triturate again; 1ml tip 10 times, 200ul tip 10 times.
13) Plate neurons, feed (1/2 media remove and replace) every 2 – 3 days.

Typical mouse neuron yield
4×10^6 neurons in cerebral cortex
71×10^6 total neurons

E17 - approx. 10^7 neurons/embryo (cortical neurons)

Hippocampal neuron dissections

1) From dissected hemispheres with the meninges removed, remove C-shaped hippocampal region into 1.5ml tube containing 0.4ml HBSS on ice.
2) Add 50uls trypsin (10x), incubate 37deg for 0.5h.
3) Spin 1000 rpm, 5' then resuspend in 1ml 1x DMEM/FBS
4) Triturate - 10 times, regular Pasteur pipette, 10 times fire polished pipette. Let tissue clumps settle 5' and transfer supernatant to new tube.
5) Count cells and distribute to coverslips in 50% Neurobasal [50% 1x DMEM/FBS; just use neurobasal only]. Spin and resuspend hippocampal neurons if necessary.

If glial contamination is a problem, culture with 2uM arabinoside at DIV4
Neurobasal is supplemented with B27, typically with penicillin/streptomycin