MEI Protocol

Cerebellar granule neuron culture and migration recording in vitro

Materials:
Coverslip (18mm in diameter, Fischer)
Poly-D-lysine (100ug/ml)
Laminin (20 µg/ml; Sigma)
Recording well and glass slides

Culture medium:
BMEM+10%FCS or NB+2%B27

In vitro recording medium:
L15 or L15+2%B27

Preparation of the coverslips
Glass coverslips were coated with PDL for 12hr and washed with sterile water for twice(the coated coverslips can be stored at -20^{0}C for 2 weeks.)
100ml Laminin was added to the PDL coated coverslip and kept at 37^{0}C for over 2 hr (we often extended the coating time to 12 hr as 2 hr seemed not enough) .
Remove the laminin and rinse the coverslip with culture medium at least for once before use. Don not let the laminin coated coverslip dry in the air.

Cell culture
1. Cerebella obtained from P0-P2 SD rat were quickly removed from the skull, and placed in cold HBSS and freed from meninges and choroid plexus.
2. The cerebella were digested in 0.5%Trypsin+0.02EDTA, 37^{0}C, for 15min. dissociated neurons was cultured at density of 0.3~$1*10^{5}$/ml. The neurons were ready for recording after 12 hr.

Migration recording:
1. Preparation of the recording well

Clean glass slides were sealed to the plastic wells by melted wax (from wax candle). The glass and well must be clean, or they will detach from each other often.

2. Migration recording

Sealed wells and recording medium were prewarmed to 37^{0}C. 1ml recording medium was added to the well, and then the coverslip with migrant neurons was added. The medium was covered with 0.5ml oil to prevent evaporation.

The well was placed on a heatable stage set at 37^{0}C, and the neurons can be recorded

for over 10 hr.
If needed, a new coverslip can be added to the well, and the oil will not touch the neurons.

After recording, the glass was always discarded and the well should be washed carefully to remove the oil. The well should not be kept in ethanol.