Acute isolation of hippocampal astrocytes protocol

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Procedure

1. Dissect brains from postnatal day 5–30 mice and cut into 300 µm thick slices in frontal orientation by using a vibratome (HM 650V; Microm International, Walldorf, Germany).
2. Perform slice preparation in an ice-cold, carbogen-saturated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) containing sucrose (pH 7.4).
3. Incubate for 30 min at 35°C in ACSF supplemented with sucrose, and store the slices for 20 min in ACSF.
4. Incubate the slices for 7–15 min in papain-containing (25 U/ml; Sigma P-4762 supplemented with L-cysteine monohydrate 0.8 mg/ml) ACSF at room temperature (bubbling with carbogen is necessary). For tissues from older mice incubate the slices for up to 15 min.
5. After washing, dissect the CA1 region of the hippocampus and isolate cells in HEPES-buffered solution using wide-bore pipettes. The cell suspensions contain many astrocytes identified by their characteristic morphology, based on the fluorescence in the case of cell dissociation from hGFAP-EGFP or Cx43kiECFO mice, or after staining of astrocytes with SR101 (1 µM; Kafitz *et al.*, 2008, *J. Neurosci. Meth*. 169:84–92).
6. Perform SR101 incubation during the ACSF/sucrose incubation step at 35°C.

Reagents

ACSF with sucrose

87 mM NaCl
2.5 mM KCl
1.25 mM NaH_2PO_4
7 mM $MgCl_2$
0.5 mM $CaCl_2$
25 mM $NaHCO_3$
25 mM D-glucose
75 mM sucrose gassed with carbogen

ACSF without sucrose

126 mM NaCl
3 mM KCl
1.25 mM NaH_2PO_4
2 mM $MgSO_4$
2 mM $CaCl_2$
26 mM $NaHCO_3$
10 mM D-glucose gassed with carbogen

HEPES-buffered solution

150 mM NaCl
5 mM KCl
2 mM $MgSO_4$
2 mM $CaCl_2$
10 mM HEPES
10 mM D-glucose gassed with O_2
Adjust solution pH to 7.4 using NaOH or HCl