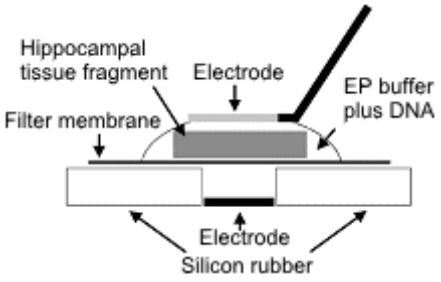
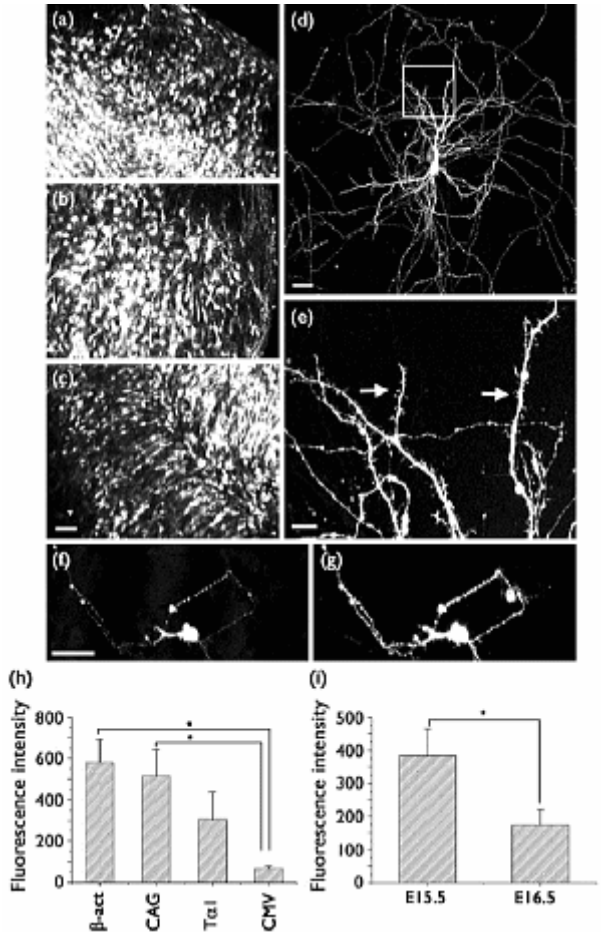
[Brain Slice] Electroporation-mediated gene transfer system applied to cultured CNS neurons



Schematic representation of an electroporation set-up.
A fragment of the mouse embryonic hippocampus was placed on a Millipore membrane filter and 5µl EP buffer containing 1mg/ml of plasmid DNA was applied onto the tissue.
A tungsten needle was attached to the surface of a droplet.
After application of square pulses the tissue fragment was returned to a petri dish containing ice-cold HBSS solution.



Electroporation-mediated expression of fluorescent proteins in hippocampal neurons.

(a-c) Organ culture of hippocampal tissue fragments three days after electroporation with CAG-eGFP (a), Tα1X4 -eGFP (b), and β-actin-eGFP (c) expression constructs.

(d, e) A mature hippocampal neuron maintained 14 days in dissociated culture after electroporation of a β-actin-eGFP expression construct. Higher magni¢cation view of the region marked by a rectangle in (d) reveals dendritic spines on the surface of dendritic shafts (arrows in e).

(f, g) A hippocampal neuron 7 days after electroporation of 1:1 mixture of Tα 1X4-eGFP and Tα1X4-mRFP1. Both eGFP fluorescence (f) and mRFP1 fluorescence (g) can be observed in a single cell.

(h) Relative fluorescence intensity of hippocampal tissue fragments after electroporation of eGFP-expression plasmids with four different promoter sequences. The tissue fragments were maintained in culture for 4 days, fixed and observed under a confocal microscope. Fluorescence intensities per unit area of the tissue fragments were determined.

(i) 2Relative fluorescence intensity of hippocampal tissue fragments isolated at two different developmental stages and electroporated with β-actin-eGFP. Tissue fragments were maintained for 4 days in culture and subsequently fixed. Fluorescence intensities were measured using a confocal microscope.

Bars = 50 μm (a-d, f, g); 10 μm (e).

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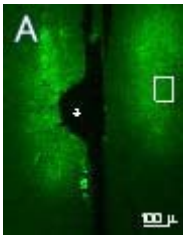
[Brain] Electroporation-mediated gene transfer in the adult rat brain



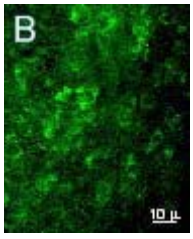
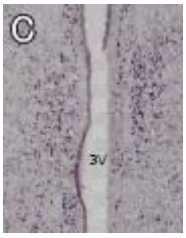


Figure A: EGFP expression in the medial preoptic nuclei of a female rat examined 4 days after bilateral electroporation at 10 weeks of age. (An asterisk indicates the trace of the positioning of the electrode)

Figure B: EGFP-positive cells (high magnification of Fig. A using a 60x objective lens). EGFP fluorescent signals are observed in the perikarya.



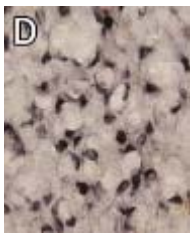


Figure C: Estrogen receptor αimmunoreactivity in the medial preoptic nuclei and the periventricular nuclei of an adult female rat.
3V: third ventricle

Figure D: Estrogen receptor α-positive cells (high magnification of Fig. C using a 60x objective lens). Estrogen receptor α immunoreactivity is prominent in the nuclei.

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