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Transfection into Mouse-Rat: BRAIN-TISSUE by Electroporation

The NEPA21 is the only device on the market to approach Electroporation from the perspective of optimising delivered energy.

- Compared to devices from other suppliers, the NEPA21 system offers the researcher a level of previously unavailable control over energy delivery to the electroporation target. This control is generated via unique electroporation pulse-output configurations, client-confirmed protocols and application-customised electrodes.
- With this market-leading control and (user-independent) reproducibility of the technique, it is now possible to apply electroporation techniques to applications previously considered too sensitive for electroporation methodologies.

- The finer control over the delivered energy offers specific and important advantages for BRAIN - TISSUE electroporation. As the thrust of NEPA21 protocols is to minimise delivered energy, this means that the targets are electroporated with less current (than competing device protocols).
- Only delivering the required energy (and no more) to porate the membrane is of utmost importance for viability post electroporation.
- The success of the NEPA21 for retina electroporation is evident by the Application and Publication information following.
- The NEPA21 system is supported by a suite of over 250 different electrode configurations, which further enhance the applicability of the system and empower researchers with further experimental options and opportunities.

APPLICATIONS

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Electroporation-mediated gene transfer in the adult rat brain

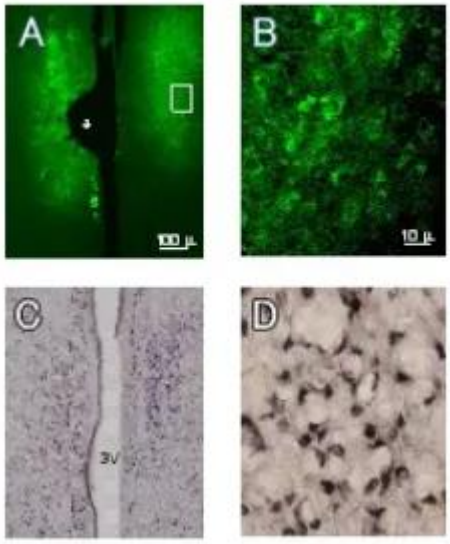
Figure A: EGFP expression in the medial preoptic nuclei of a female rat examined 4 days after bilateral electroporation at 10 weeks of age. (An asterisk indicates the trace of the positioning of the electrode)

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 immunoreactivity is prominent in the nuclei.

Tetsuo Shirakawa, Center for Advanced Oral Medicine, Hokkaido University Hospital



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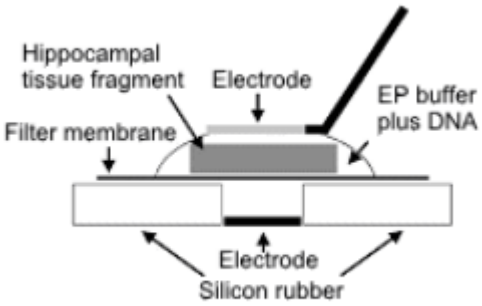
Electroporation-mediated gene transfer system applied to cultured CNS neurons



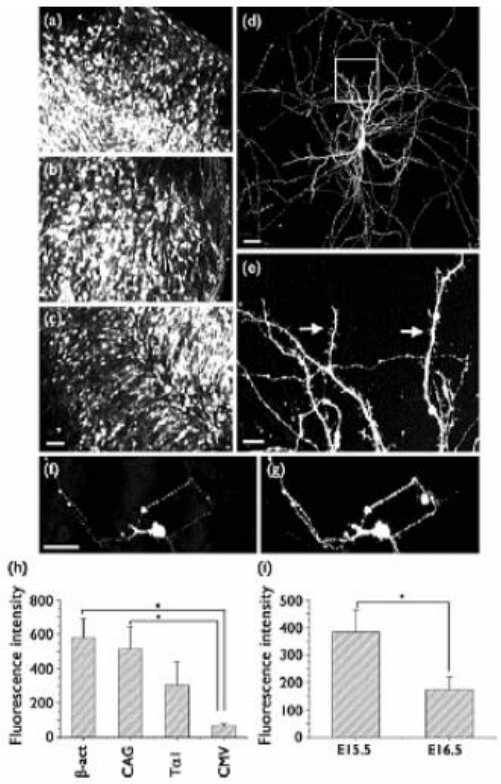
Schematic representation of an electroporation set-up.

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) Ta1X4 -eGFP, (b) and b-actin-eGFP, (c) expression constructs.

(d,e) A mature hippocampal neuron maintained 14 days in dissociated culture after electroporation of a -actin-eGFP expression construct. Higher magnification 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 Ta1X4-eGFP and Ta1X4-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 b-actin-eGFP. Tissue fragments were maintained for 4 days in culture and subsequently fixed. Fluorescence intensities were measured using a confocal microscope.

Shigeo Okabe, Department of Cellular Neurobiology, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo
***Neuroreport, Volume 15, Issue 6, Pages 971-975, April 29, 2004**

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PUBLICATIONS

Transfection into Mouse-Rat: BRAIN-TISSUE by Electroporation

Cerebellum lobe culture / P5_mice

Nesprin-2 coordinates opposing microtubule motors during nuclear migration in neurons
Zhou, C., Wu, Y. K., Ishidate, F., Fujiwara, T. K., Kengaku, M.
J Cell Biol. 2024 Nov 4;223(11):e202405032.

P0_mouse_brains / Forebrain

Genome editing of Nf1, Pten, and Trp53 in neonatal mice induces glioblastomas positive for oligodendrocyte lineage transcription factor 2
Yamamoto H, Yamamura K, Nagasaki H, Suzuki T, Ninomiya F, Matsubara K, Harada N, Ohkubo S.
J Toxicol Pathol. 2021 Oct;34(4):359-365.

P2_mouse_brains / P21_mouse_brains

Primary cilium-dependent cAMP/PKA signaling at the centrosome regulates neuronal migration
Stoufflet J, Chaulet M, Doulazmi M, Fouquet C, Dubacq C, Métin C, Schneider-Maunoury S, Trembleau A, Vincent P, Caillé I.
Sci Adv. 2020 Sep 2;6(36):eaba3992.

P0_mouse_brains

Rho Family GTPases, Rac and Cdc42, Control the Localization of Neonatal Dentate Granule Cells During Brain Development
Ito H, Morishita R, Mizuno M, Tabata H, Nagata KI.
Hippocampus. 2019 Jul;29(7):569-578.

P2_Mouse_brains

A dual role for the transcription factor Sp8 in postnatal neurogenesis
Gaborieau E, Hurtado-Chong A, Fernández M, Azim K, Raineteau O.
Sci Rep. 2018 Sep 28;8(1):14560.

Organotypic brains slices / E15.5

Precise Somatotopic Thalamocortical Axon Guidance Depends on LPA-Mediated PRG-2/Radixin Signaling
Cheng J, Sahani S, Hausrat TJ, Yang JW, Ji H, Schmarowski N, Endle H, Liu X, Li Y, Böttche R, Radyushkin K, Maric HM, Hoerder-Suabedissen A, Molnár Z, Prouvot PH, Trimbuch T, Ninnemann O, Huai J, Fan W, Visentin B, Sabbadini R, Strømgaard K, Stroh A, Luhmann HJ, Kneussel M, Nitsch R, Vogt J.
Neuron. 2016 Oct 5;92(1):126-142.

Cortical slices / P0_hamster_brains

Cortical excitatory neurons become protected from cell division during neurogenesis in an Rb family-dependent manner.
Oshikawa M, Okada K, Nakajima K, Ajioka I.
Development. 2013 Jun;140(11):2310-20.

Cortical slices

Wnt/calcium signaling mediates axon growth and guidance in the developing corpus callosum.
Hutchins BI, Li L, Kalil K.
Dev Neurobiol. 2011 Apr;71(4):269-83.

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New born mouse brain

Cellular Mechanismhs Underlying Morphine Analgesic Tolerance and Hyperalgesia
Hiroshi Ueda
Opioid-Induced Hyperalgesia, First, Pages 9-20, October 2009

P0_P4_mouse_brains

Efficient In Vivo Electroporation of the Postnatal Rodent Forebrain
Boutin C, Diestel S, Desoeuvre A, Tiveron MC, Cremer H.
PLoS One. 2008 Apr 2;3(4):e1883.

New born brains

Plexin-A2 and its ligand, Sema6A, control nucleus-centrosome coupling in migrating granule cells
Renaud J, Kerjan G, Sumita I, Zagar Y, Georget V, Kim D, Fouquet C, Suda K, Sanbo M, Suto F, Ackerman SL, Mitchell KJ, Fujisawa H, Chédotal A.
Nat Neurosci. 2008 Apr;11(4):440-9.

Adult Mouse brains

Genetic manipulation of adult mouse neurogenic niches by in vivo electroporation
Barnabé-Heider F, Meletis K, Eriksson M, Bergmann O, Sabelström H, Harvey MA, Mikkers H, Frisén J.
Nat Methods. 2008 Feb;5(2):189-96.

P8 mouse brain

Microtubule-based nuclear movement occursindependently of centrosome positioning in migrating neurons
Umeshima H, Hirano T, Kengaku M.
Proc Natl Acad Sci U S A . 2007 Oct 9;104(41):16182-7.

Cerebellar slices

Homer proteins control neuronal differentiation through IP(3) receptor signaling.
Tanaka M, Duncan RS, McClung N, Yannazzo JA, Hwang SY, Marunouchi T, Inokuchi K, Koulen P.
FEBS Lett. 2006 Nov 13;580(26):6145-50.

Cultured telencephalic hemisphere

The Caudal Migratory Stream: A Novel Migratory Stream of Interneurons Derived from the Caudal Ganglionic Eminence in the Developing Mouse Forebrain
Yozu M, Tabata H, Nakajima K.
J Neurosci. 2005 Aug 3;25(31):7268-77.

Embryo / Hippocampus

Electroporation-mediated gene transfer system applied to cultured CNS neurons
Kawabata I, Umeda T, Yamamoto K, Okabe S.
Neuroreport . 2004 Apr 29;15(6):971-5.

Adult mouse brains

Locus-Specific Rescue of GluR1 NMDA Receptors in Mutant Mice Identifies the Brain Regions Important for Morphine Tolerance and Dependence
Inoue M, Mishina M, Ueda H.
J Neurosci. 2003 Jul 23;23(16):6529-36.

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Adult rat brains

Up-regulation of Protein-disulfide Isomerase in Response to Hypoxia/Brain Ischemia and Its Protective Effect against Apoptotic Cell Death

Tanaka S, Uehara T, Nomura Y.

J Biol Chem. 2000 Apr 7;275(14):10388-93.