Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

Consortium leader

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

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Neurobiológia alapjai - Módszerek

BASICS OF NEUROBIOLOGY - Methods

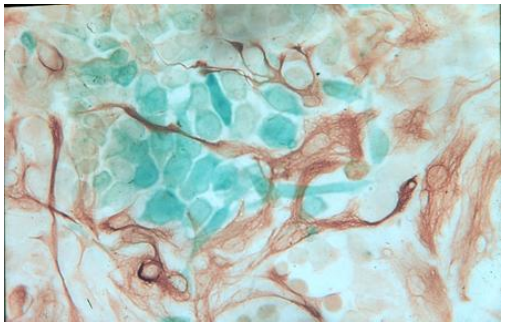
By Imre Kalló

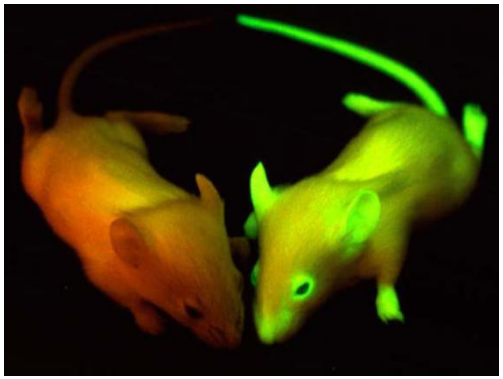
METHODS IN NEUROBIOLOGY VI.

Living experimental models

Imre Kalló
Pázmány Péter Catholic University, Faculty of Information Technology

I. Histology techniques: light microscopic studies
II. Applications using fluorescent dyes
III. Histology techniques: electron microscopic studies
IV. Techniques to map neuronal connections
V. Molecular biological techniques
VI. Living experimental models
VII. Electrophysiological approaches
VIII. Behavioral studies
IX. Dissection, virtual dissection, imaging techniques





LIVING EXPERIMENTAL MODELS

In vitro experimental objects used for studying the nervous system

Explant cultures (from early embryonic tissue)
Organotypic slices (from embryonic and early postnatal tissue)
Primary cultures (from embryonic and early postnatal tissue)
Immortalised cell lines
Embryonic **S**tem Cells (ES cells)

In vivo experimental objects used for studying the nervous system

Intact animals
Animals underwent various treatments
Genetically modified animals
Humans

IN VITRO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM

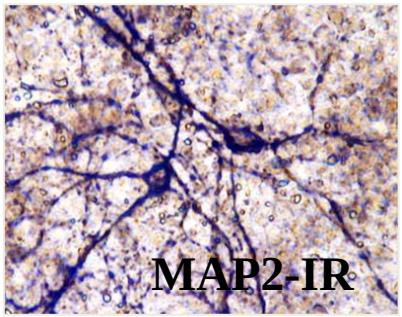
Why do we need *in vitro* approaches to study the nervous system?

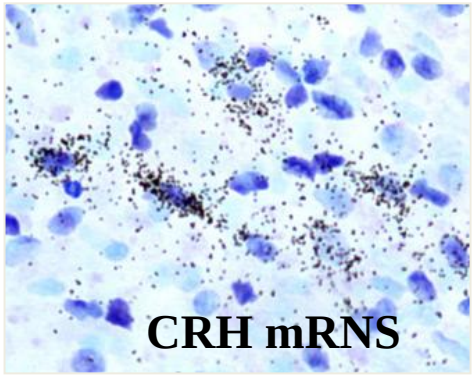
- The nervous system is the most complex organ of vertebrates; the estimated number of neurons and glial cells in the mammalian brain is about 10^{11}-10^{13}, which can be morphologically and functionally very different. By using *in vitro* experiments for testing these cells at multiple conditions, the number of animal sacrifice can be minimized!
- Studies on the biochemical and molecular biological processes of single cells are limited *in vivo*. *In vivo*, there are limitations also for testing the effects and the operational mechanism of drugs influencing the function of nervous system (pharmacology).
- Certain cell types are present in low number in the nervous system, or they do not knit in a compact nucleus, instead they are scattered in a larger area in the brain; characterisation of the cellular processes of these neurons *in vivo* is rather complicated.

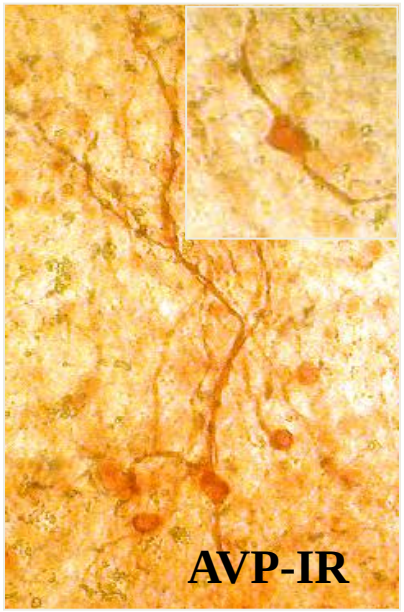
Limitations of *in vitro* studies

- Positional interactions characterising the *in vivo* conditions are absent or present only in a limited extent and can be partially reproduced at *in vitro* conditions.
- Consequently, interpretation of data from the arteficial *in vitro* conditions must be evaluated and perceived with caution!

IN VITRO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Organotypic slices

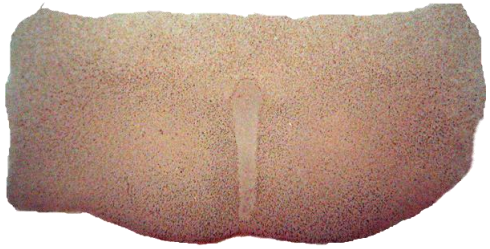






Characteristics of slices:

- differentiated cells
- local neuron-neuron or neuron-glia connections are kept
- remote neuronal input is lost
- intrinsic networks may remain functional
- easy access, “targeting potential”
- pharmacological studies

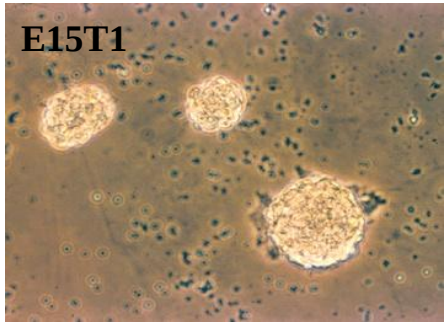


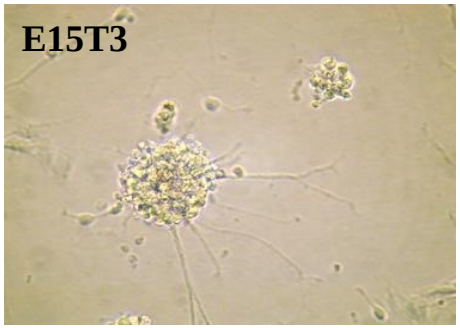
IN VITRO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Primary cultures

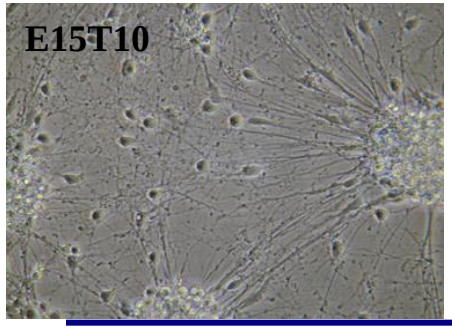
Source of cells:

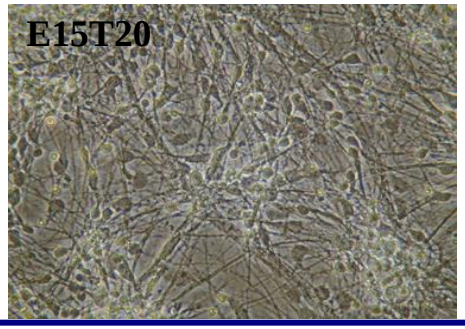
Embryo: neuronal and glial progenitor cells (retinoic acid induction)

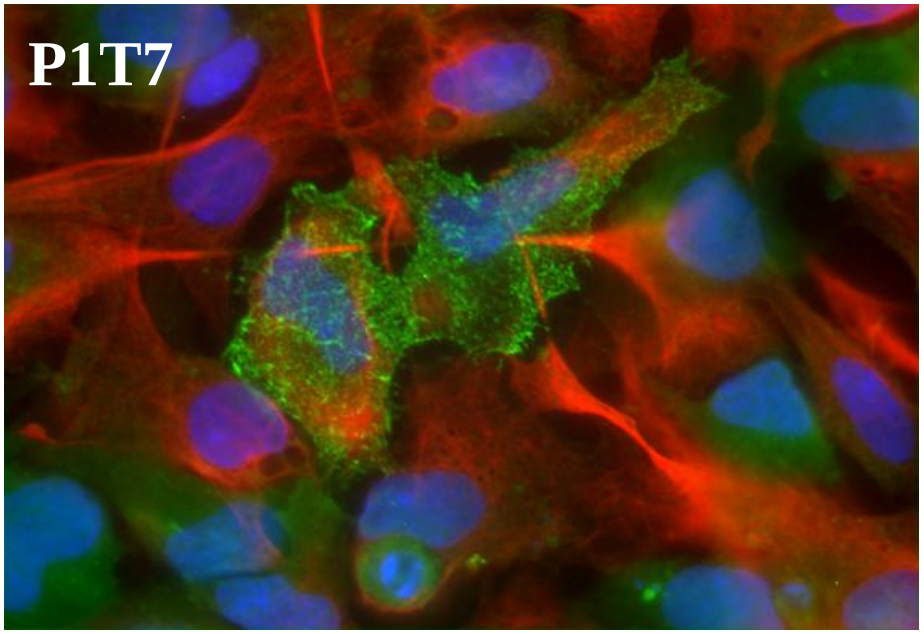
Newborn animals: mainly glial cells (neurons die off shortly after preparation – they do not divide at this age!)

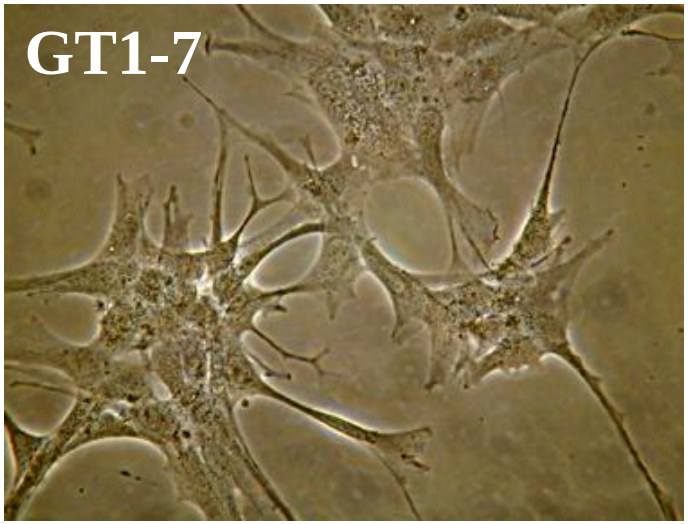


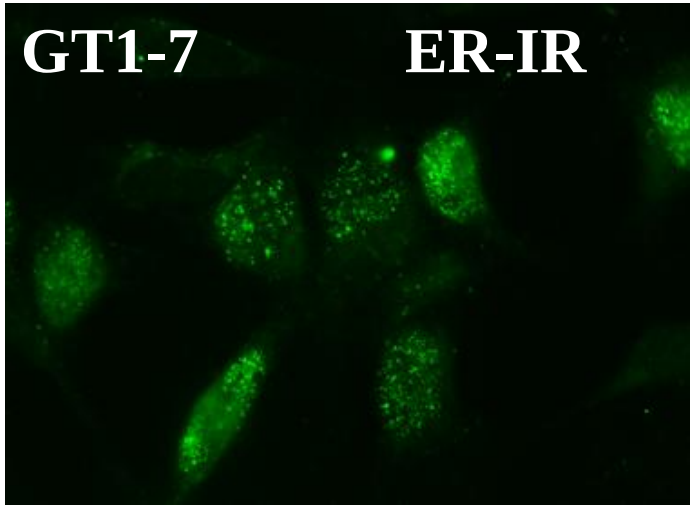






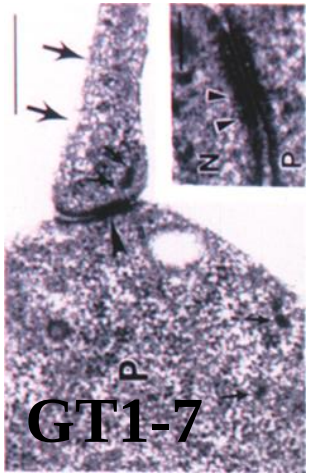


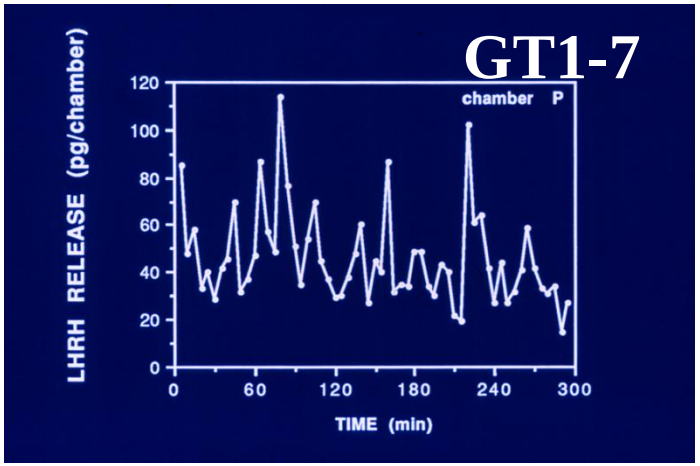




IN VITRO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Immortalised cell lines

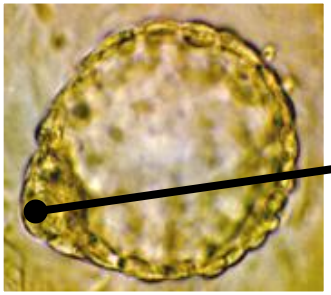
Definition: Cell lines are group of cells, in which the daughter cells (clones) are morphologically and functionally identical, they are capable to renew their colony (they are capable to divide infinitely). By their divisions two identical daughter cells are produced with identical developmental potentials.



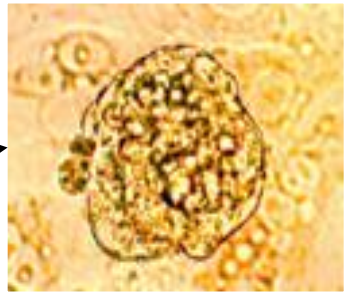


IN VITRO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Embryonic Stem Cells

Mouse



Blastocyst

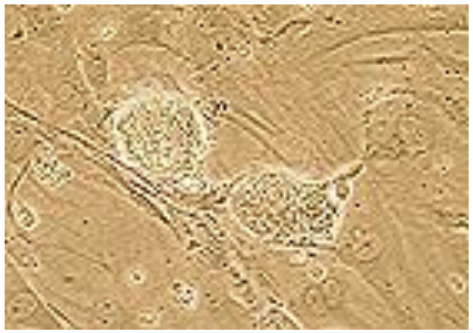


Inner cell mass (ICM)

LIF

feeder

Culture

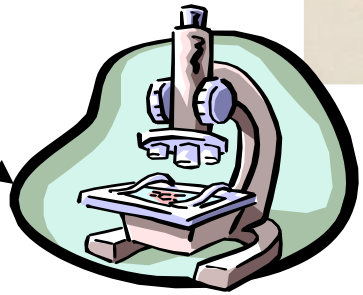


Embryonic stem cells

GENETIC MODIFICATION

Mouse

Cardiomyocytes
Endothelial
Melanocytes
Neurons
Glial cells
Pancreatic islet cells
Primitive endoderm



IN VIVO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Intact animals

Factors to be considered:

Species differences exist – data obtained from one species can not be considered to apply in 1:1 to other species (e.g. localization of neuronal phenotypes differs)

There are also **strain differences** - data obtained from one strain can not be considered to apply in 1:1 to other strains (e.g. mice stains with high or low nocturnal melatonin levels)

The **same strain** from different vendors may **show differences**

Gender and **age differences** are very significant!

There are significant **individual differences** – characterization of a population is needed!

The physiological state (and the function of nervous system!) of the animals show **seasonal, infradian, circadian (**e.g. diurnal vs nocturnal**), ultradian changes**!

The **laboratory conditions** determine the responses of animals given to a challenge (e.g. temperature, availability a food, running wheel, social partners – stress, aggression, court, nurse etc.)

IN VIVO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Animals underwent pharmacological or surgical treatments

Aims of the pharmacological treatments:

To anaesthetize the animals – effect of anesthetics on the activity of neurons must be taking in consideration (e.g. EEG changes of sleeping animals)

To change **systemically** or **selectively the function of CNS cells** (e.g. receptor agonists)

Route of the pharmacological treatments (significance of the hepatic clearance and the BBB):

Systemically: subcutaneously (sc), intravenously (iv), intraperitoneally (ip)

Locally: intracerebroventricularly (icv), in the extracellular space of the brain, intracellularly

Aims of the surgical treatments:

To alter the hormonal/physiological status of the animal (e.g. gonadectomy)

To deliver drugs/recording/stimulating tools into target areas – according to 3D coordinates of **stereotaxic instruments** (e.g. injecting tracer molecules)

To obtain/implant embryos, tissues, cells (e.g. implantation of embryonic stem cells)

Important!

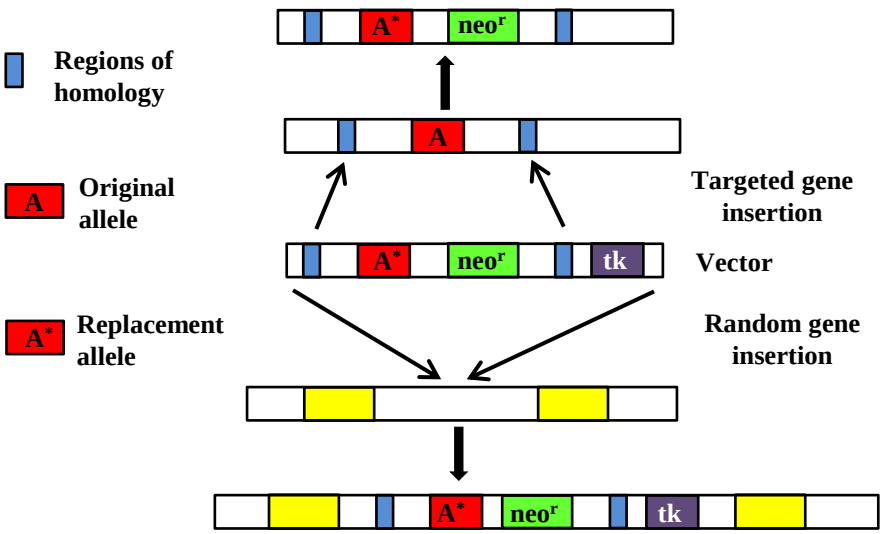
Neither of the interventions are allowed to cause any unnecessary pain, suffering of the animals!

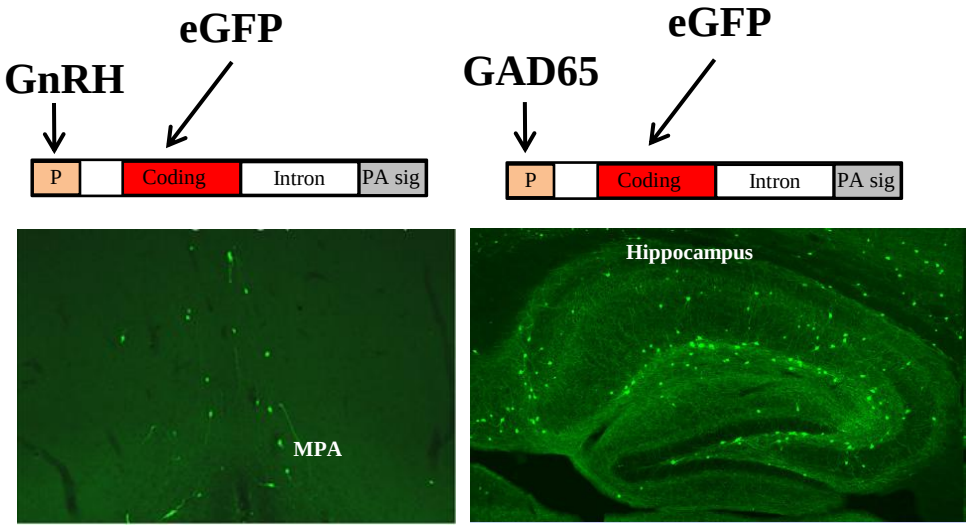
IN VIVO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Genetically modified animals

Transgenic animal

Aims:

Gene-therapy
Over-expression of genes
Introducing a dominant negative costruction
(e.g. production of truncated proteins)
Inserting antisense RNA producing cDNA in the genome
Expression of strange genes (e.g. eGFP)
KO (″loss-of -function″) and
KI (″gain-of -function″)





IN VIVO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Genetically modified animals

Cell-specific production of transgens: the CRE-LoxP system

1. Production of transgenic animal stock, cell-specifically expressing the ″CRE” enzyme. (problems of the promoter specificity and strength)
   or generating viral constructions encoding the ″CRE” enzyme. 

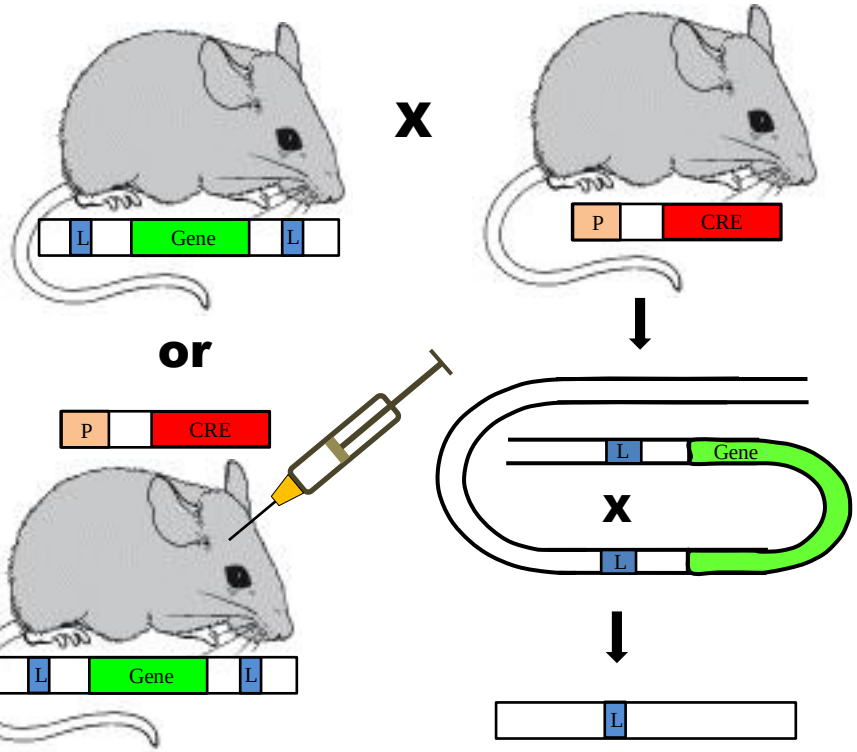
2. Production of conditional KO or KI animal stocks (all introns contain loxP sites (L))
   

3. Cross breeding the two animal stocks or infecting defined regions of the brains with viral vectors carrying the construct for the ″CRE” recombinase.

Cell-specific recombination!

Knock-out (KO) animals



IN VIVO EXPERIMENTAL OBJECTS USED FOR STUDYING THE NERVOUS SYSTEM: Humans

Limited access to living brain tissue (strictly licensed procedure only to obtain pathological tissue i.e. epileptic focus or brain tumor)

Renaissance of electric field potential recordings (prediction of seizure, controlling robotic devices, deep brain stimulation etc.)

Imaging techniques in the current forefront of the diagnostic and research activities! (CT, PET, PET-CT, SPECT, MR, fMRI**)**