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PPCU FIT ● fall semester 2015

BASICS OF NEUROBIOLOGY

Methods

WRITTEN EXAM

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Important information

Dear Candidate!

This booklet is made for the Written Exam in Basics of Neurobiology. Please note, that this booklet contains only the "Methods" chapter which is only a part of the exam!

In some cases you may find errors or typos in the text. If this happens please, feel free to report them on my website.

We wish you a successful preparation!

Edited by:
Márton Bese NASZLADY– 2016

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Topics

I. Histology techniques: light microscopic studies

Histology: Techniques based on *in vivo* or *post mortem* sampling.

Preservation of the sample

- Heat (microwaves)
- Chemical preservation (perfusion or immersion)

Cutting sections

Precise cutting requires embedding and sharp knives. Widely used cutting tools:

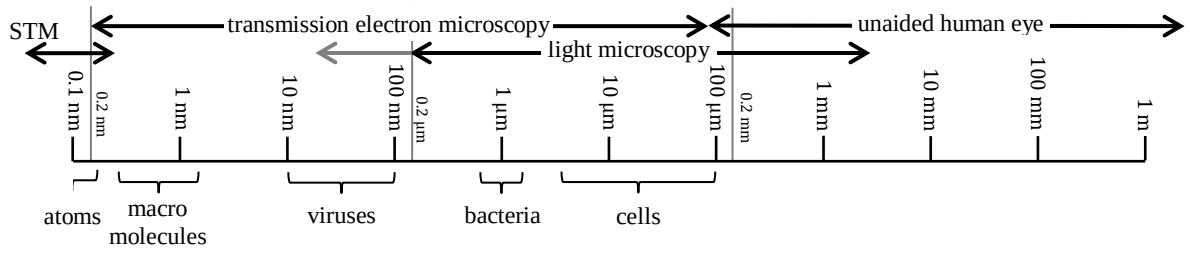
– Scalpel, razor blade – free hand cutting	200 – 50 µm
– Microtome	5 – 20 µm
– Freezing microtome	10 – 50 µm
– Vibratome, vibroslicer	20 – 300 µm
– Criostat	10 – 300 µm
– Ultratome	40 – 80 nm

Staining techniques

Staining is required in order to localize and identify tissue components, cells, cell organelles or molecules. Techniques:

- **Histochemistry**
  Dyes – binding to molecules according to their physico-chemical properties. Proteins, lipids, carbohydrates, nucleic acids can be visualized.
- **Enzyme histochemistry**
  The principle is that a first, enzyme + substrate reaction leads to a product, which can be demonstrated by a second reaction. Enzymes can be acidic an alkaline phosphatases, dehydrogenases etc.
- **Autoradiography**
  Binding or incorporation of radioactively labeled ligands, molecules.
- **Immunohistochemistry**
  Detection of molecules with antigenic properties: direct and indirect labelling; enzyme, fluorescent labels; intensification techniques.
- **In-situ hybridization histochemistry**
  Detection of DNA, heteronuclear and messenger RNA: riboprobes, oligoprobes, radioactively labelled probes and non-radioactively labelled probes.
- **Detection of transgenic reporter-proteins**

Observation possibilities



Light microscope

Principle: Light coming from an illumination source goes through the condenser lens then the specimen. The image is made by the objective and projective lenses and can be viewed through the ocular.

There are several types of light microscopy:

- **Bright Field Microscopy**
  All the light goes through the specimen and forms an image.
- **Dark Field Microscopy**
  A specially sized disc blocks direct light from the light source, only the light scattered from the sample enters the objective lens, while the directly transmitted light is not collected.
- **Phase Contrast Microscopy**
  Image contrast is improved in two steps: background light is phase shifted $-90°$ and the background is dimmed by a gray filter ring.
- **Differential Interface Contrast Microscopy**
  This method uses polarized light and special prisms to create the image
- **Fluorescence Microscopy**
  Light coming from an appropriate illumination source excites the fluorescent dye in the tissues. The emitted light is reflected by a dichroic beam splitter and the image is created from this reflected beam after filtering the illumination wavelength.
- **Confocal Laser Microscopy**
  Based on fluorescence microscopy. Light coming from the beam splitter goes through a pinhole therefore only the light coming from the focal point will be detected.
- **Two-Photon Microscopy**
  Dyes in the tissue absorb two, small energy (infrared) photons almost concurrently. Uses two dichroic beam splitters, green and red wavelengths are detected. Advantages: no fading, no phototoxicity, increased focal depth.
- **Super-Resolution Microscopy**
  Takes several images from the sample and uses mathematical computations to enhance the image.
- **Stochastic Optical Reconstruction Microscopy**
  A type of Super-Resolution Microscopy uses stochastic approaches in image enhancement.

II. Applications using fluorescent dyes

Fluorescent molecules absorb their own characteristic wave-length of light, which turn them into a higher energy state (excitation) and upon returning to the lower energy state, they emit light (emission), the wave-length of which characterizes the molecule again. Two important properties: *absorption maximum* and *emission maximum*.

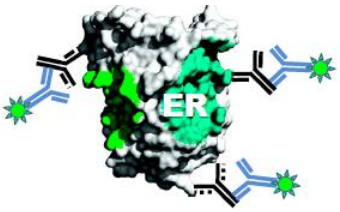
Fluorescent molecules are widely used as:

- **selective marker** of cellular organelles and tissue components
- labels of reagents and immunoglobulins in **immunocytochemistry**
- labels of probes in **in situ hybridization histochemistry**
- **sensors** of intracellular calcium levels and potential changes
- **reporter molecules** expressed by genetically altered cell types of CNS

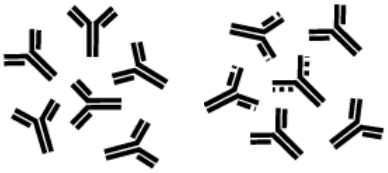
Fluorescent immunohistochemistry (FIHC)

Antigen detection

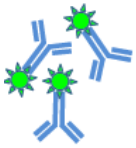
Immunofluorescent detection of estrogen receptors (ERs)



Using monoclonal or polyclonal immunoglobulins as *primary antibody* (PAB)



Applying fluorescently-labelled immunoglobulins as *secondary antibody* (SAB)

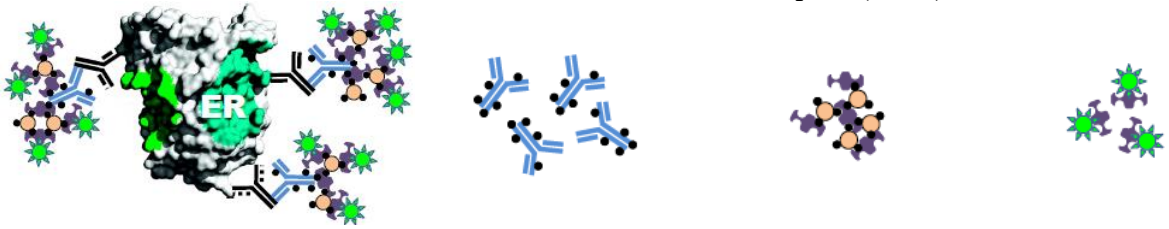


Signal amplification technique using the avidine-biotin system

After PAB using biotinylated immunoglobulins

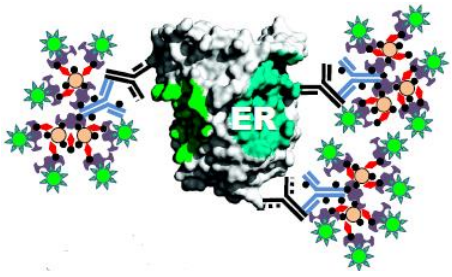
And Avidine and Biotinylated peroxidase enzyme Complex (ABC)

And either of the fluorescently labelled avidine

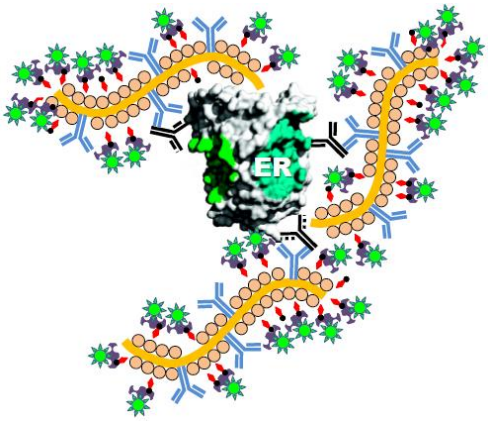


Signal amplification technique using the biotinylated-tyramine system

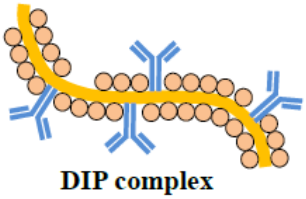
By reacting with H_2O_2 and Biotinylated Tyramine (BT), the peroxidase enzyme of the ABC deposites BT



Signal amplification technique using the dextran-immunoglobulin-peroxidase (DIP) and the biotinylated-tyramine systems



By reacting with H_2O_2 and Biotinylated Tyramine (BT), the the DIP complexes deposites BT



Fluorescent in situ hybridization (FISH)

The aim is to detect a single stranded mRNA in the tissue. We use antisense RNA probes labelled either at the 3' end with digoxigenin/biotin or throughout with biotinylated nucleotides. After hybridization the digoxigenin and biotin are detected with specific antibodies, which then are revealed with simple or amplified fluorescence techniques.

Calcium imaging

Physiology

Calcium ions are kept intracellularly at nanomolar concentrations (100 nM). Elevations of which from the extracellular space (1.2 mM) and intracellular stores change the membrane potential, as well as activate calcium-dependent intracellular processes. These elevations can be investigated in fluorescent or two-photon confocal microscopy. Slow, moderate and rapid changes can be distinguished.

Calcium indicators

- Chemical indicators (lipophilic molecules, which includes fura-2, indo-1, fluo-3, fluo-4 and Calcium Green-1) loaded in the cells
- Genetically encoded indicators (fluorescent proteins fused with calmodulin, which includes Pericams and Cameleons) expressed in specific subpopulations of cells

Usage

Stimulated cells either loaded with the indicator or expressing the indicator are viewed in a fluorescence microscope or a two-photon confocal microscope. Images are captured by a CCD camera (data acquisition at rates 10–100 ratios/sec; 30-200 msec/image required) and analyzed according to intensity.

Voltage sensitive dyes

Physiology

The membrane potential is a voltage difference generated by the altered ionic concentrations on the opposite sides of the cellular membrane. Profiles of propagating action potentials and subthreshold potentials can be monitored directly with voltage-sensitive dyes.

Voltage indicators

Voltage-sensitive dyes are organic molecules or proteins. They reside in a cell membrane and change their optical properties in response to a change in membrane potential. Slow dyes and fast dyes are distinguished for practical reasons (e.g. ANEP dyes, ANNINE-6plus).

Usage
With fast (1 kfps frames rate) cameras voltage-sensitive dyes can monitor membrane potential in processes of individual neurons and from multiple cell bodies in localized brain regions.

Genetic engineering to introduce fluorescent markers

Transfection is the introduction of gene sequences encoding GFP, YFP, CFP or BFP into eukaryotic cells using viral vectors, electroporation etc. After these proteins were expressed within the cells, they can be visualized with fluorescent microscopy.

Förster resonance energy transfer

A donor chromophore transfers energy to an acceptor chromophore – if they close enough (typically less than 1 nm) to each other – through non-radiative dipole-dipole coupling. FRET reporters are used to study protein-protein and protein-DNA interactions as well as protein conformational changes.

III. Histology techniques: electron microscopic studies

Transmission Electron Microcopy

Principle: Electron beam coming from an illumination source goes through the condenser lens then the specimen. The image is made by the objective and projective lenses and can be viewed on the fluorescent screen.

Electron density: In the electron microscope, electrons are projected onto ultrathin sections of the sample. Electrons, which permeate the sample, generate a lighter area on the screen underneath by exciting its fluorescent coating. Many of the electrons are, however scattered by the dense regions of the sample never reaching the screen and resulting in dark areas on it. Consequently, a 2D image of the sample is appearing with increasingly darker areas on the screen (and the electron micrograph), where the sample is correspondingly more „electron dense".

Preparation of the samples

Standard work

1. Chemical fixation at RT
2. Dehydration
3. Embedding in resins
4. Ultracutting
5. Contrasting

High resolution work

1. Cryo-fixation followed by either
   - observation on a cryo-stage
   - cryo ultratomy
   - freeze fracture (FF)
   - **freeze substitution** (FS) (diddolution of ice by an organic solvent + fixative)
2. Embedding

Scanning Electron Microcopy

Principle: The SEM scans the surface of the sample with a 2-3 nm diameter high-energy electron beam, which interacts with the surface atoms of the sample and generates secondary electrons, back-scattered electrons, x-rays, light, reflected electrons, specimen current and transmitted electrons. Image is created most commonly by detecting secondary electrons.

The scanning electron microscope generates high resolution images (details in a few nm range) with characteristically large depth of field, which supplies the images with 3D appearance.

Preparation of the samples

1. Cryo-fixation or Chemical fixation
2. Dehydration – critical point drying (solvents, transitional fluid – carbon-dioxide)
3. Sputter coating (gold, gold/palladium) in case of non-conducting samples.

Freeze fracture – studying membranes, membrane particles in replicas

The freeze-fracture technique consists of physically breaking apart (fracturing) a frozen biological sample; structural detail exposed by the fracture plane is then visualized by vacuum-deposition of platinum-carbon to make a replica for examination in the transmission electron microscope. The five key steps in making a freeze-fracture replica are (i) cryo-fixation, (ii) fracturing, (iii) coating, (iv) etching and (v) immunolabelling.

After fracturing the extracellular side of the lipid double-layer will be the P surface and the inner side is called the E surface.

Electron-tomography

Electron tomography is an extension of traditional transmission electron microscopy. In the process, a beam of electrons is passed through the sample at incremental degrees of rotation around the center of the target sample. This information is collected and used to assemble a three-dimensional image of the target.

IV. Techniques to map neuronal connections

Correlated light microscopy and electron microscopy processing

Transection of neuronal pathways – neurodegeneration
After cutting fibers of a neuron the cell degenerates. Degenerating cells and the neighboring immunereactive cells can be visualized.

Multiple labelling techniques

Using neuronal tracers

Single cell filling and reconstruction
Filling a single cell with dye (e.g. neurobiotin) leads to a clear image where the cell can be easily distinguishable from the background.

Tracing neuronal pathways (anterograde, retrograde)
Studying the afferent and efferent connections of a cell group of the brain by using anterogradely and/or retrogradely transported tracers.

Application of transneuronal tracers

Gap junctions – Lucifer Yellow
The immortalized GnRH synthetizing (GT1-7) cells establish often gap-junctions with each other, through which the tracer Lucifer Yellow injected in one cell will fill up also the coupled one.

Chemical synapses – neurotropic viruses
Virus-infected neurons will die after time and the infection propagates towards the coupled neurons. If the virus contains fluorescent proteins, confocal laser microscopic images can be taken where infected neurons are expressing the fluorescent protein.

Latest approaches

High-Resolution Molecular Imaging Tools

Brainbow
The Cre/lox recombination was used to create a stochastic choice of expression between three or more fluorescent proteins (XFPs). Integration of tandem Brainbow copies in transgenic mice yielded combinatorial XFP expression, and thus many colors, thereby providing a way to distinguish adjacent neurons and visualize other cellular interactions.

Rainbow transsynaptic viral tool
Multiple, colored PRVs used to demonstrate spatial organization of parallel circuits. Different regions are labelled transsynaptically with different colored Rainbow viruses.

Automated Electron Microscopy

Automated imaging of ultra-thin sections at high resolution:

- Serial Section Transmission Electron Microscopy (SSTEM)

Automated imaging of sequentially exposed block faces with excellent section-to-section image registration and full automation of operation:

- Serial Block Face Scanning Electron Microscopy (SBFSEM)
- Backscattered electron scanning electron microscopy (BsSEM)
- Focused Ion Beam Scanning Electron Microscope (FIBSEM)

V. Molecular biological techniques

Studies on Gene Expression

Northern blot

It is a technique, which examines gene expression via RNA samples blotted onto membranes. It has low sensitivity and it is well quantifiable. Steps:

1. Isolation of total or mRNA
2. Size-dependent separation in gel
3. Membrane-blotting
4. Hybridization on membrane – with probes labelled isotopically or non-isotopically; re-hybridization with other probes is limited

Polymerase Chain Reaction (PCR)

It is a DNA amplification technique, which is capable to multiply a single or a few copies of a piece of DNA by several orders generating thousands to millions of copies. It has very high sensitivity; quantification only after very strict calibration. Steps:

1. Isolation of DNA sequences
2. Amplification with heat-resistant polymerase (Taq, Vent etc.) in the presence of gene-specific oligonucleotides
3. Size-dependent separation of the reaction product in agarose gel

Reverse Transcriptase PCR (RT-PCR)

It is a technique which is commonly used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA.

1. Isolation of total RNA – synthesis of cDNA sequences
2. Amplification with heat-resistant polymerase (Taq, Vent etc.) in the presence of gene-specific oligonucleotides
3. Size-dependent separation of the reaction product in agarose gel

DNA Chip Technology (DNA Microarray)

Steps:

1. Isolation of total RNA (maybe mRNA selection)
2. Synthesis of cDNA labeled with fluorochrome by reverse transcriptase
3. Hybridization of labeled probe with the chip
4. Computer-based evaluation of the fluorescence signal

Comparison of expression pattern of different samples by concurrent examination of several thousands of genes: “high-throughput”

Gene Sequencing

Gene sequencing refers to methods, by which the order of nucleotide bases – adenine, guanine, cytosine, and thymine – in the DNA molecule can be determined.

Next Generation Sequencing (NGS)

NGS includes several new approaches to reduce the time required for sequencing.

Studies of Proteins

Western blot

It is a technique, which examines proteins separated by electrophoresis and blotted onto membranes. Sensitivity is dependent on antibodies available. This technique is quantifiable, therefore a "low-throughput" method.

Proteomics

The genome of an organism's is more or less constant, the proteome differs from cell to cell and from time to time!
Proteomics is a technique, which examines proteins separated by electrophoresis and analyzed by Matrix-Assisted Laser Desorption/Ionization (MALDI) and Time-Of-Flight mass spectrometry. High sensitivity, critical point is control selection. "High-throughput" method.
Steps:

1. Proteins undergone post-translational modifications
   - phosphorylation – dephosphorylation
   - ubiquitination
   - methylation, acetylation, glycosylation, oxidation and nitrosylation
2. Proteolysis

VI. Living experimental models

In vitro experimental objects used for studying 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; characterization of the cellular processes of these neurons in vivo is rather complicated.

Limitations of in vitro studies
Positional interactions characterizing 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 artificial in vitro conditions must be evaluated and perceived with caution!

Explant cultures (from early embryonic tissue)

Organotypic slices (from embryonic and early postnatal tissue)
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

Primary cultures (from embryonic and early postnatal tissue)
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!)

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.

Embryonic Stem Cells (ES cells)

In vivo experimental objects used for studying the nervous system

Intact animals
Factors to be considered:

- **Species differences** exist – data obtained from one species cannot 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 cannot be considered to apply in 1:1 to other strains.
- The same strain from different vendors may show differences
- Gender and age differences are very significant!
- 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.)

Animals underwent various 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!

Genetically modified animals

Aims:

- Gene-therapy
- Over-expression of genes
- Introducing a dominant negative construction (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")
- KI ("gain-of –function")

Cell-specific production of transgenes: 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.

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)

VII. Electrophysiological approaches

Electrophysiology is the study of electrical properties of biological cells and tissues.

In vivo electrophysiology

Anesthetized and moving animals

Measuring field potentials

The field potential is the summation of spatial and temporal alterations of synaptic and voltage-dependent currents in a defined region of the brain. Consequently, it refers to and characterizes the activity of a certain cell or afferent population. Different methods have different names:

- From the surface of the skull (EEG)
- From the surface of the brain (ECoG)
- From the brain (LFP, MUA)
  - Tetrodes allow to visualization of single neurons from different positions.
  - Local Field Potential (generated by membrane currents) input of the cells
  - Multi Unit Activity (spiking of local neurons) output of the cells

Multiunit recordings

Single cell recordings

Juxtacellular recording

It is an extracellular recording technique. It makes a correlated electrophysiological and morphological examination possible. Electrode and marker of cell filling (neurobiotin). Fine positioning of the electrode – changes of the amplitude of action potentials. Current impulse (1-10 nA, anode impulse) – modulation of neuronal activity. Duration of filling: 15-20 min

Intracellular recording

In vitro electrophysiology

Patch-clamp recording

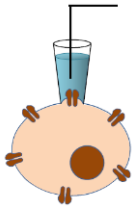
Voltage clamp

The cell's potential is clamped at a chosen value. The size of ionic current crossing the cell's membrane at the chosen value is measured.

Current clamp

The current clamp technique records the membrane potential by injecting current into a cell through the recording electrode. The membrane potential generated by the cell „spontaneously" or in response to external stimulus is measured.

Loose patch, Cell-attached, Whole cell, Perforated patch modes



Cell-attached mode

Transfer is only through ion channels entrapped

Whole cell mode

Content of the pipette redistribute with that of the cytoplasm

Perforated patch mode

Ion transfer through the perforations, but large molecules stay within the cytoplasm

VIII. Behavioral studies

Circadian behavior

Activity is monitored in running wheel. Overexpression of $VPAC_2$ in the mouse SupraChiasmatic Nucleus (SCN) generates a change in the circadian rhythm of the animal – free-running period is decreased!

Measuring physiological parameters eating and drinking behavior

Eating
Animal models are developed to study pathological weight gain and weight loss.
Can be measured: circadian time and frequency of eating, calorie intake, running wheel activity, basal metabolic rate etc.
Cannot be measured: evaluation of self-image

Drinking
Patients are drinking a lot, and urinating a lot (10 liters or more)! Cause: absence of the effect of Anti Diuretic Hormone (ADH) in the kidney. Animal model is the Brattleboro rat. Mutation in the gene encoding vasopressin (ADH).
Can be measured: circadian time and frequency of drinking, drinking volume and amount of urine

Reproductive behavior

Male sexual behavior: mounting
Determined by the testosterone surge at early postnatal period!
If there is no testosterone effect in the brain at the critical period, sexual behavior will be feminized – male pups will show lordosis behavior. Caused by: gonadectomy; lack of androgen receptor mediated effects (ARKO, Tfm)

Female sexual behavior: lordosis
Absence of testosterone surge at early postnatal period!
If testosterone injected into the animal in the critical period (postnatal 3-5 days), sexual behavior will be masculinized – female pups will show mounting behavior.

Social interaction: social interaction, dominant and subordinate animals

Pheromones of dominant males induces social preference and increased neurogenesis in the subventricular zone (SVZ) and dentate gyrus (DG) of female mice.

Behavioral tests of fear and anxiety

Open field
This test is based on conflicting innate tendencies of avoidance of bright light and open spaces (that ethologically mimic a situation of predator risk) and of exploring novel environment.

Light/dark box
The Black and white test (also named light-dark test) is based on the conflict of natural tendencies of rodents to avoid lighted and open areas and to explore novel environments.

Hole board
The animal is placed on an arena with regularly arranged holes on the floor. Both frequency and duration of spontaneous elicited hole-poking behavior are then measured during a short period of time.

Vogel-test
The Vogel test paradigm is a popular conflict model in which water-deprived rats and mice first learn to lick from a water spout in an operant chamber.

Then, usually after a period of unpunished licking, responses are punished with mild foot-shocks, inducing a significant reduction of drinking. In this context, administration of anxiolytics is shown to inhibit shock-induced drinking suppression.

Elevated plus maze
The test that is based on two conflicting innate tendencies: exploring a novel environment and avoiding elevated and open spaces constituting situations of predator risk.

Social interaction
This test consists in allowing the experimental subject freely exploring an unfamiliar congener in its homecage or in a neutral environment. Social exploration is measured by the time spent by the experimental subject around the congener as well as the amount and duration of behaviors that compose social interaction (e.g. sniffing, following, allogrooming, biting, mounting, wrestling…).

Passive avoidance
Passive avoidance is fear-motivated tests used to assess short-term or long-term memory. During the acquisition phase the animal is placed in the white compartment and when the animal crosses to the black compartment it receives a mild foot shock. Thus the animal learns that the moving to the dark compartment has negative consequences.

Others…

Behavioral tests of depression

Chronic mild stress

Bulbectomy

Flinder's sensitive line

Sleep deprivation

Cognitive tasks – spatial memory

Morris watermaze
Used for testing spatial memory. The tank is about 2 m wide with non-transparent water in it, within the swimming rat has to find a sub water level platform by using the laboratory furniture and other objects visible from the tank as orientation landmarks. After random swimming, the rat learned about the position of the platform, and swims to it immediately from any point of the tank.

Correlated electrophysiology and behavioral tests premotor neurons encode the goal of movement

Correlated electrophysiology and behavioral tests prediction of future

IX. Dissection, virtual dissection, imaging techniques

Dissection

Mortui docent vivos

Classical way of dissection – in situ demonstration of the anatomical and pathological details in their 3D appearance – for specialist

New preservation technique – plastination makes the whole body or body parts possible to preserve for long time and to demonstrate them with safety – also for public

New approach – high resolution maps of the human body accessible through user friendly softwares

New elements – human atlas of 2-8mm-thin slices cut from epoxy embedded bodies in the major planes of space – to support the new imaging techniques

Viva docent vivos

Classical way of imaging – 2D projection -, reflection -, emission images of the body or body parts from a single direction

"New" imaging techniques – based on tomography - gathering projection data from multiple directions

Modern tomography – combined with high speed computation to process, analyze, combine, store images and make 3D reconstructions of the body or body parts. X-ray CT MR PET SPECT

New approach in neuro research – new tools to investigate population of neurons activated by defined cognitive tasks.

Imaging techniques

Photography	Radiation	Electromagnetism
Microscopy	X-ray	MRI, fMRI
Optical imaging	SPECT	(MEEG)
	PET	(BEAM)
	Autoradiography	

PET

PET uses pre-produced radioactive ligands, C-11, N-13, O-15 or F-18 isotopes. After radioligand is injected the PET scanner takes the image. With different ligands different biochemical/physiological parameters can be measured:

- Blood flow, blood volume; metabolism (oxygen, glucose, amino acids, fatty acids, fluorine etc.) receptor systems (dopaminergic, cholinergic, serotoninic, benzodiazepin-GABA, opioid etc.) pharmacodynamics and pharmacokinetics, protein synthesis, molecular diffusion and tissue pH.

MRI

Proton spins of the subject in the big magnetic field (B_0) are aligned parallel or antiparallel with the direction of the field. Radio waves are transmitted into the subject [for about 3 ms] – perpendicular to B_0, the magnetic component (B_1) of this electromagnetic wave temporarily knocks the protons out of alignment. Radio wave transmitter is turned off, while it receives radio waves re-transmitted by the protons "relaxing" back to their undisturbed "equilibrium" position. Retransmitted radio waves are manipulated with magnetic fields during this readout interval [10-100 ms]. Storing of measured radio wave data vs. time-repetitions of transmission- reception and processing raw data leads to the reconstructed image.

fMRI

Physiological parameter measured:
Increased neuronal activity requires more glucose and oxygen, which is rapidly delivered through the blood stream resulting in local changes in cerebral blood volume (CBV) and cerebral blood flow. These in turn generate local changes in the relative concentration of oxygenated (HBO_2) and deoxygenated hemoglobins (HBr), which serves as the Blood Oxygen Level Dependent signal (BOLD), a marker for MRI (hemoglobin is diamagnetic when oxygenated, but paramagnetic when deoxygenated.