



**PETER PAZMANY
CATHOLIC UNIVERSITY**



**SEMMELWEIS
UNIVERSITY**



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

Consortium leader

PETER PAZMANY CATHOLIC UNIVERSITY

Consortium members

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Nemzeti Fejlesztési Ügynökség

ÚMFT infóvonal: 06 40 638 638

nfu@nfu.gov.hu • www.nfu.hu

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ELECTROPHYSIOLOGICAL METHODS OF THE STUDY OF THE NERVOUS- AND MUSCULAR SYSTEM

Az ideg- és izomrendszer elektrofiziológiai vizsgálómódszerei

LECTURE 5

PROPERTIES AND CHARACTERISTICS OF ELECTRODES

(Elektródok tulajdonságai, jellemzői)

DOMONKOS HORVÁTH, GYÖRGY KARMOS

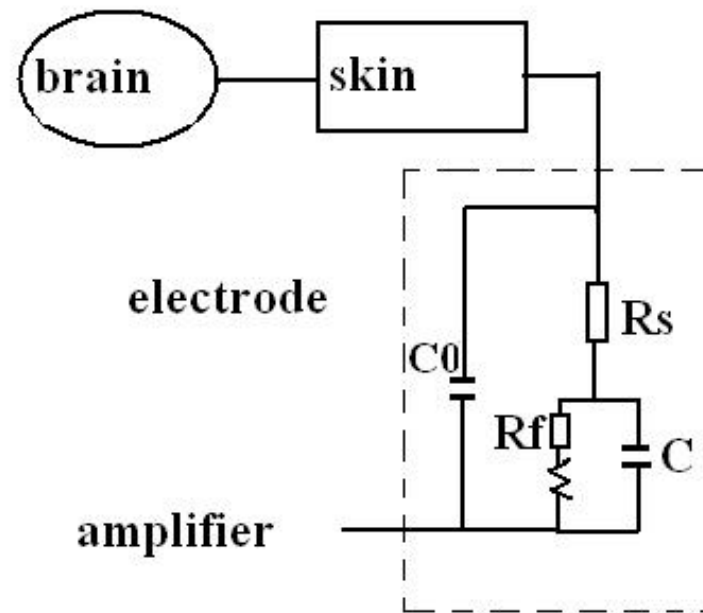
BIOPOTENTIAL RECORDINGS

- **Biopotential recording:** connection between recording object (inanimate) and living structure
- **Recording object:** electrode, i. e. electron conductor or complex of an electron conductor and an electrolyte
- In an electron conductor, charge is conducted by electron movement
- In an electrolyte, charge is conducted by charged particle (ion) movement
- In a recording, always at least two electrodes is used because always potential difference between two electrodes is measured
- **Electrode potential:** potential energy measured on an electrode. Only theoretical definition, this is a potential compared to an arbitrary zero electrode potential, the potential of a standard hydrogen electrode

GENERAL PROPERTIES OF RECORDING ELECTRODES

- **Ideal electrode does not exist:** electrodes cannot have ideal signal transmission characteristics. Living tissue is a chemically aggressive medium for electrodes: there is always a chemical reaction between the electrode and the tissue
- **Electrode impedance:** many contributing factors, including electrode material, electrolyte type, etc.
- **Electrode potential:** when electrode is placed in a conducting solution a potential difference can be measured between the electrode and the bulk of the solution
- **Electrode stability, biocompatibility:** on the one hand, electrodes have to withstand the chemically aggressive living tissue, on the other hand, electrode-caused harm to the living tissue has to be minimized

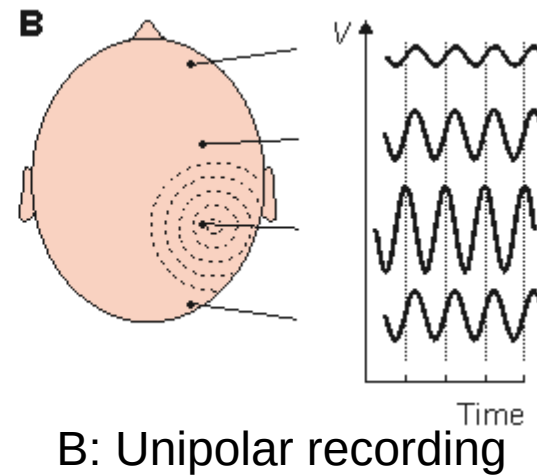
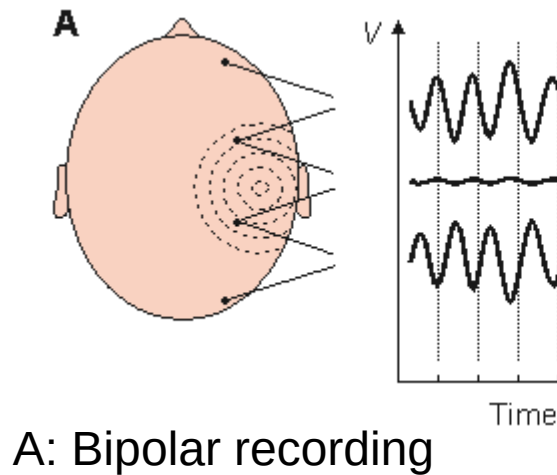
REPLACEMENT DIAGRAM OF ELECTRODE IMPEDANCE



- R_s : electrode paste resistance
- R_f : electrode resistance
- C : electrode capacitance
- $\approx$: Wartburg resistance – frequency dependent
- C_0 : stray capacitance

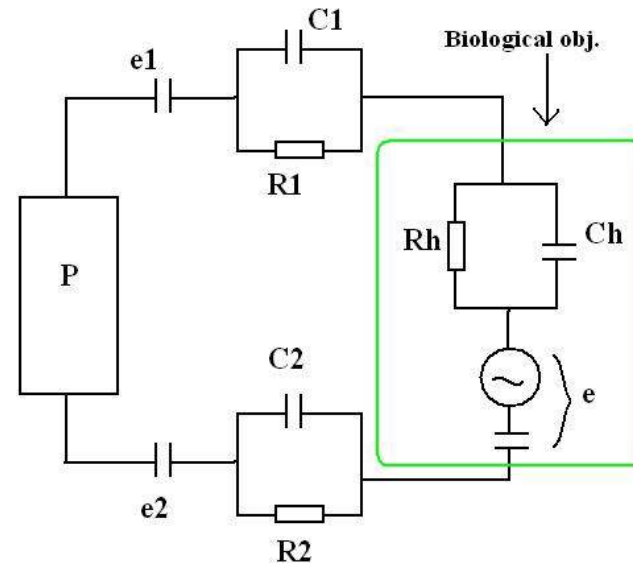
TYPES OF BIOPOTENTIAL RECORDING

- **Bipolar recording:** Both electrodes are in active tissue. Signal of an active electrode is compared to a neighboring active electrode as zero potential.
- **Unipolar recording:** there is an indifferent electrode in inactive tissue as a zero potential electrode. The signal of active electrode is compared to this zero potential.



Source: <http://www-psych.nmsu.edu/~jkroger/lab/principles.html>

REPLACEMENT DIAGRAM OF BIPOLAR RECORDING



e : Biological signal(V)

R_H : Scalp resistance

C_H : Scalp capacitance

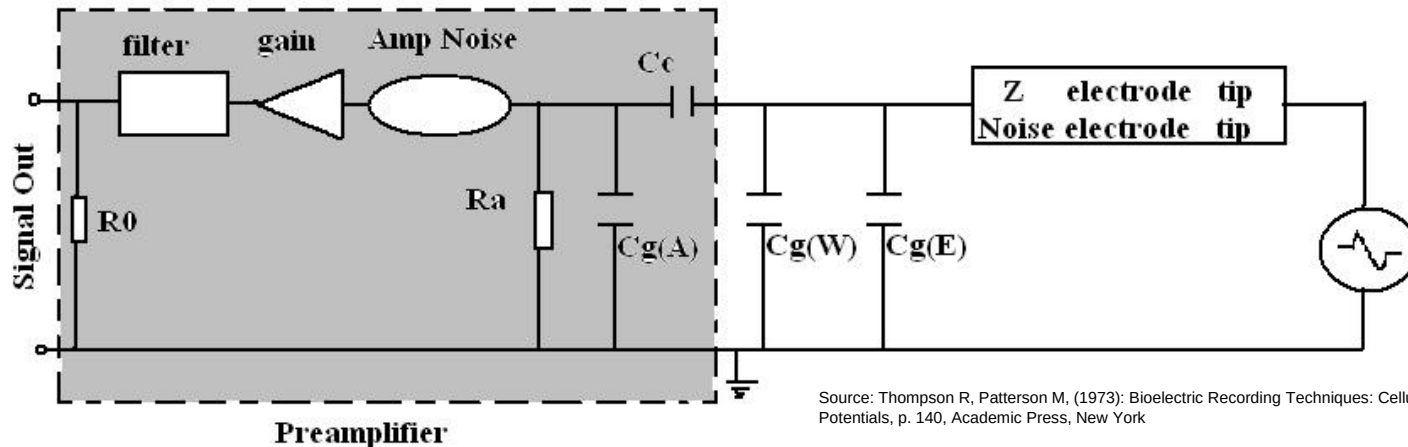
e_1 and e_2 : Electrode potentials (V)

R_1 and R_2 : Electrode resistances

C_1 , C_2 : Electrode capacitances

P : Amplifier

REPLACEMENT DIAGRAM OF MICROELECTRODE RECORDING



Source: Thompson R, Patterson M, (1973): Bioelectric Recording Techniques: Cellular Processes and Brain Potentials, p. 140, Academic Press, New York

Z : electrode tip impedance

$C_{g(E)}$: electrode-ground capacitance

$C_{g(W)}$: wire-ground capacitance

$C_{g(A)}$: amplifier input-ground capacitance

C_g : connection capacitance

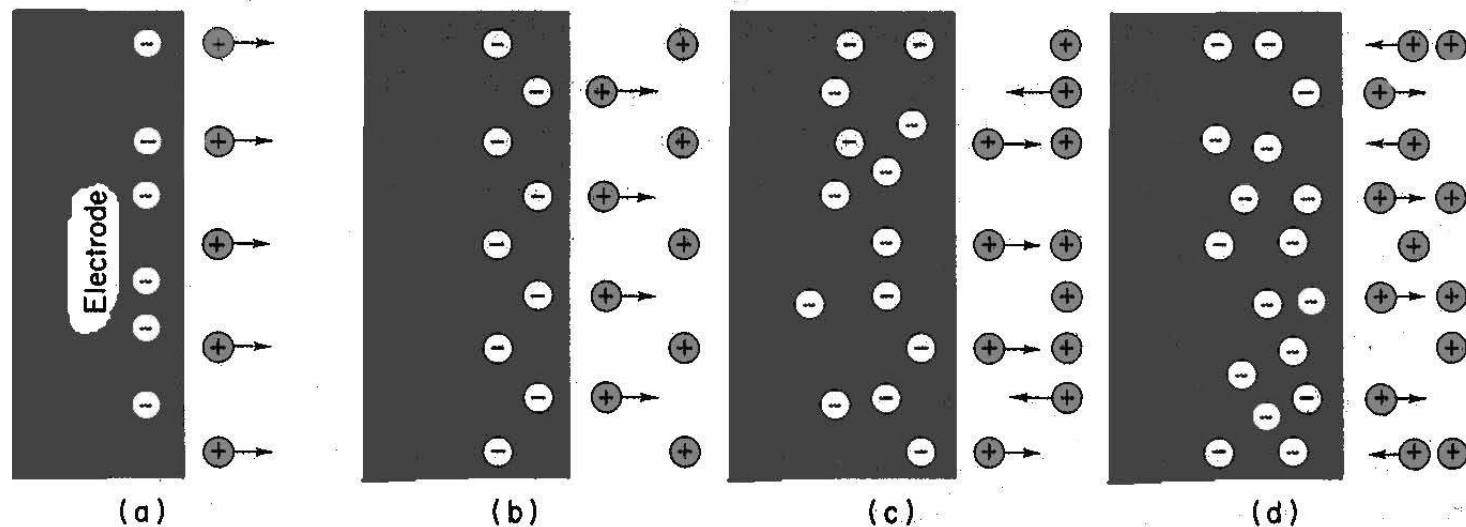
R_a : amplifier input impedance

R_0 : amplifier output impedance

The above figures show how many different factors can contribute to signal distortion measured on an electrode. Capacitive factors make changes frequency dependent while input and output impedance mismatch influence signal amplitude.

ELECTRODE POTENTIAL

At a metal-liquid junction ion movement develops that forms polarization. As a result of ion movement between metal and solution balance sets up between the two mediums. This forms the electrode-electrolyte double layer.



Source: Cooper R, Osselton JW, Shaw JC (1969): EEG Technology, 2nd ed., p. 21, Butterworths, London

Forming of double layer: (a) ions enter the solution after immersion of metal (b) number of ions increase in solution (c) in the metal electrode negative potential develops, ion flow out of and into the metal have different amount (d) balance sets up when the two flows are equal. Accumulated positive ions in solution form double layer.

EFFECTS OF ELECTRODE POTENTIAL

- Different metals and solutions have different impedance values due to different ion movement between them.
- Some examples for impedance:
 - Copper: $1.7\mu\Omega\text{cm}$
 - 3M KCl: $5\Omega\text{cm}$
 - Seawater: $22\Omega\text{cm}$
 - Physiological solution: $70\Omega\text{cm}$
 - Tap water: $0.2\text{-}10\Omega\text{cm}$
 - Distilled water: $1\text{-}5\text{M}\Omega\text{cm}$
- Impedances of axonic parts:
 - Membrane impedance: $10\text{-}100\text{k}\Omega\text{cm}^2$
 - Membrane capacitance: $0.5\text{-}1\text{m}\Omega\text{cm}^2$
 - Intracellular medium impedance: $50\text{-}200\Omega\text{cm}$

GALVANIC CELL FORMED BY METAL-LIQUID-METAL JUNCTION

The galvanic cell formed by the metal-liquid-metal junction follows the electrochemical series. Electrode potential is compared to an arbitrary zero electrode potential, the standard hydrogen electrode potential. The electrochemical series shows potential differences of different metal electrodes compared to standard hydrogen electrode potential. Metals with positive electrode potential are called precious metals. In comparison of two metals the more positive is called the more precious of the two.

Electrode	Electrode reaction	E^0/V
Au Gold	$Au^{3+} + 3e^- \rightleftharpoons Au$	+1.43
Ag Silver	$Ag^+ + e^- \rightleftharpoons Ag$	+0.80
Cu Copper	$Cu^{2+} + 2e^- \rightleftharpoons Cu$	+0.34
→ H Hydrogen	$H^+ + e^- \rightleftharpoons H$	0
Pb Lead	$Pb^{2+} + 2e^- \rightleftharpoons Pb$	-0.13
Sn Tin	$Sn^{2+} + 2e^- \rightleftharpoons Sn$	-0.14
Ni Nickel	$Ni^{2+} + 2e^- \rightleftharpoons Ni$	-0.25
Cd Cadmium	$Cd^{2+} + 2e^- \rightleftharpoons Cd$	-0.40
Fe Iron	$Fe^{2+} + 2e^- \rightleftharpoons Fe$	-0.44
Zn Zinc	$Zn^{2+} + 2e^- \rightleftharpoons Zn$	-0.76
Ti Titanium	$Ti^{2+} + 2e^- \rightleftharpoons Ti$	-1.63
Al Aluminium	$Al^{3+} + 3e^- \rightleftharpoons Al$	-1.66
Mg Magnesium	$Mg^{2+} + 2e^- \rightleftharpoons Mg$	-2.37
Na Sodium	$Na^+ + e^- \rightleftharpoons Na$	-2.71
K Potassium	$K^+ + e^- \rightleftharpoons K$	-2.93
Li Lithium	$Li^+ + e^- \rightleftharpoons Li$	-3.05

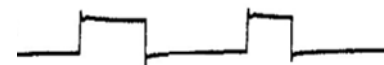
IMPORTANCE OF METAL-LIQUID JUNCTION CHARACTERISTICS: SIGNAL TRANSFER CHARACTERISTICS OF ELECTRODES

According to the electrochemical series every electrode polarizes differently when immersed into a solution. This polarization determines the signal transfer characteristics of the electrode. Note high fidelity transfer of silver/silver chloride electrode due to its lack of polarization. Other types of metal electrodes distort signal severely.

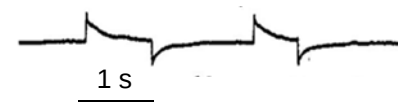
Input waveform:



Non polarizable electrode (Ag/AgCl):



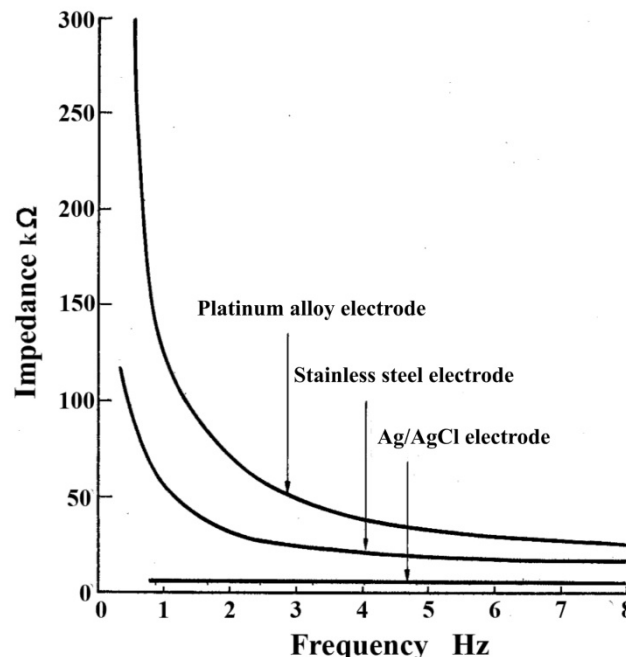
Metal electrode:



Source: Cooper R, Osselton JW, Shaw JC (1969): EEG Technology, 2nd ed., p. 27, Butterworths, London

FREQUENCY DEPENDENCE OF ELECTRODE IMPEDANCE

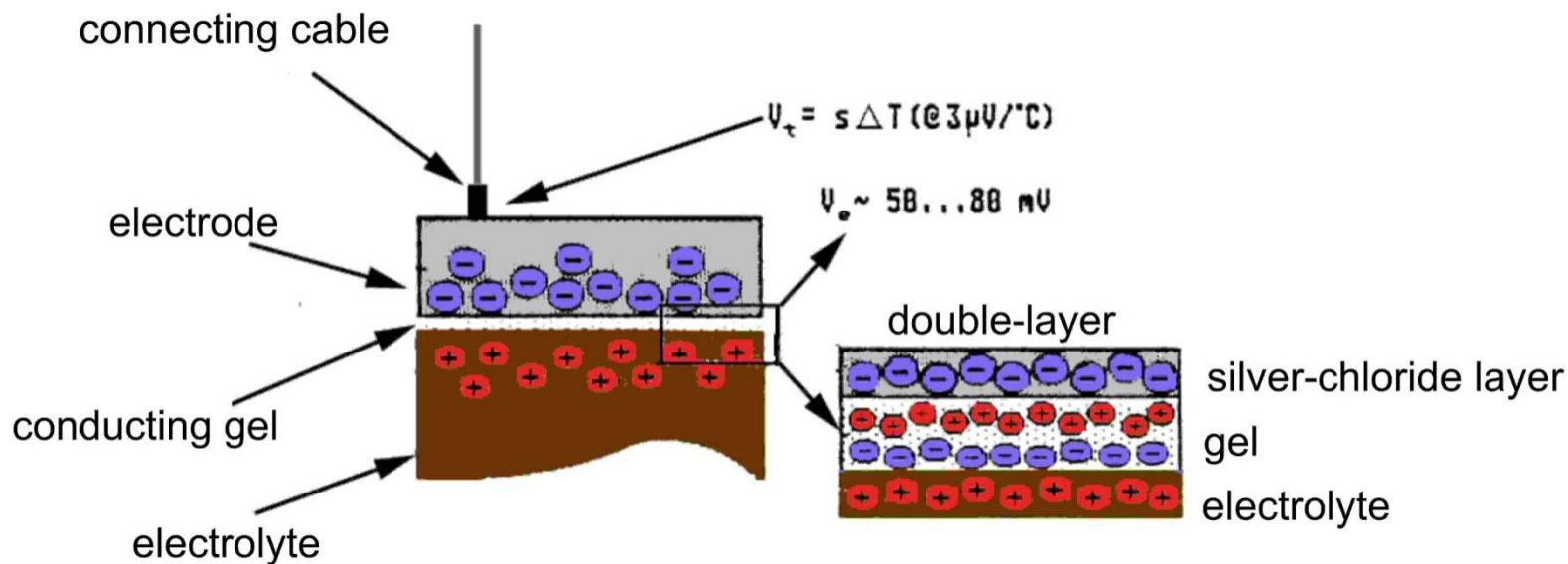
Electrode impedance changes with frequency due to capacitive properties of electrode-electrolyte double layer. At low frequencies electrode impedance rapidly increases with decrease of frequency. This can distort low-frequency recordings such as EEG recordings.



Source: Cooper R, Osselton JW, Shaw JC (1969): EEG Technology, 2nd ed., p. 28, Butterworths, London

PROPERTIES OF ELECTRODE-GEL-SKIN JUNCTION

In order to improve recording characteristics conductive gel used between electrode and skin. This gel helps forming and contributes to stability of double layer as shown on figure.

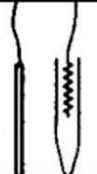
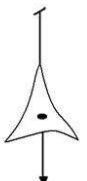
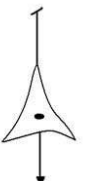


ELECTRODE TYPES USED FOR BIOPOTENTIAL RECORDINGS

- Intracellular
 - Micropipette
 - Sharp microelectrode
 - Patch-clamp electrode
- Extracellular
 - Micropipette
 - Single microwire
 - Tetrode and multielectrode microwire
 - Silicon-based multielectrodes
- Macropotential
 - Surface recordings
 - Silver/Silver chloride electrode
 - Intracerebral recordings
 - Metal microwire
 - Multielectrode
 - Needle electrode

TYPES OF BIOPOTENTIAL RECORDINGS

For every purpose different types of electrode needed. Electrodes can be placed on the scalp, on or under the dura mater surface, or even implanted into the living tissue, depending on the desired type of signal.

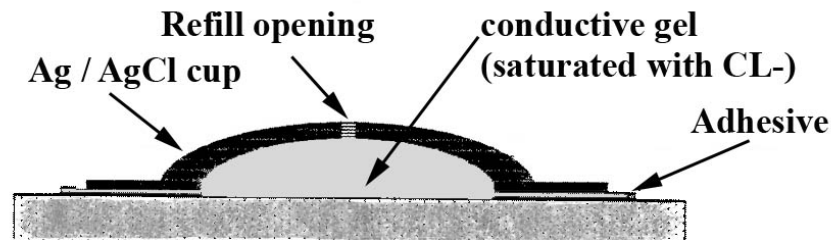
Place of recording	SCALP	EPIDURAL	SURFACE	EXTRA-CELLULAR	INTRA-CELLULAR
Recorded signal	EEG & AEP	ECoG & EP	ECoG & EP	FP & Spike	RP, SP & Spike
					
					
Area of recording surface	2-10 mm ²	1-3 mm ²	1-3 mm ²	1-50 μ ²	0.5-3 μ ²
Signal amplitude	10-200 μV	0.1-4 mV	0.2-5 mV	0.4-20 mV	1-100 mV

Source: Thompson R, Patterson M, (1973): Bioelectric Recording Techniques: Cellular Processes and Brain Potentials, p. 140, Academic Press, New York

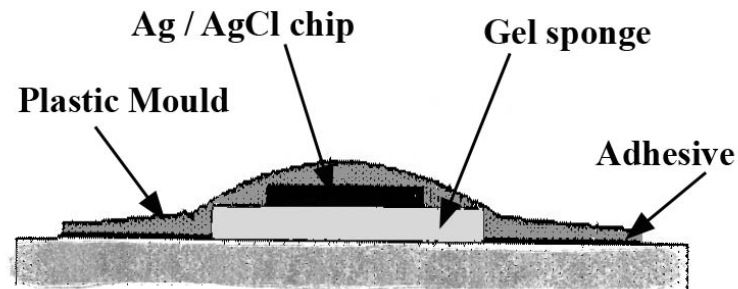
TYPES OF SILVER/SILVER CHLORIDE SCALP ELECTRODES

There are two types of silver-silver chloride scalp electrodes: reusable and single-use. Reusable electrodes have a cavity under the silver/silver chloride-cup for conductive gel and a refill opening to refill gel.

Reusable electrode

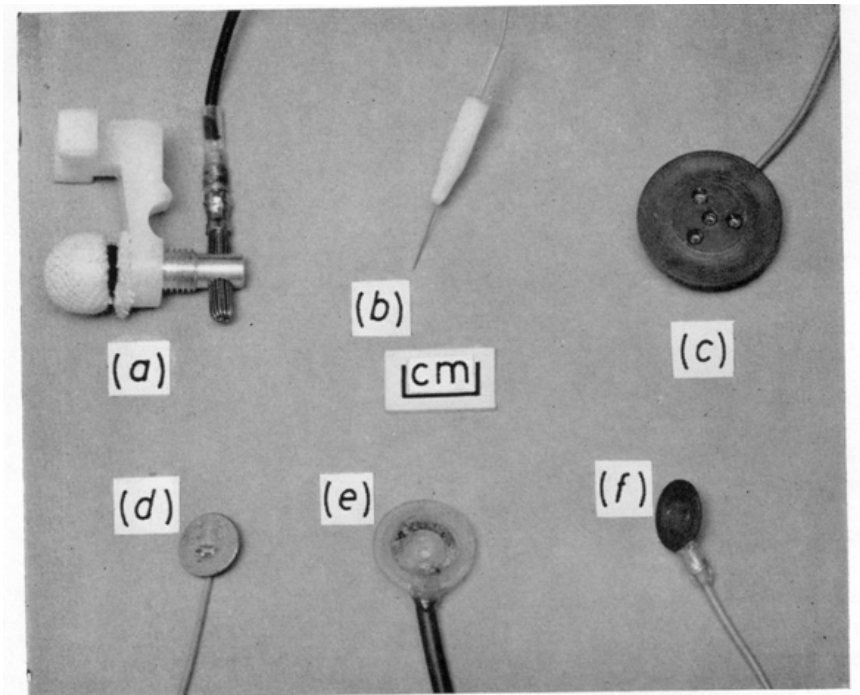


Single-use electrode



TYPES OF CONVENTIONAL EEG ELECTRODES

- Electrode pad (a)
- Subdermal needle electrode (b)
- Gold disc electrode (d)
- Chlorided silver wire in plastic cup (c,e)
- Silver/silver chloride cup (f)



Source: Cooper R, Osselton JW, Shaw JC (1969): EEG Technology, 2nd ed., Plate II, Butterworths, London

TYPES OF EEG ELECTRODE NETS

In order to gain more information from a recording more electrodes are used at the same time: electrode nets are built. Two basic types:

1. Electro-cap

- Allows skin preparation
- Application in 30 minutes
- Used with most EEG amplifiers
- Disinfection (glutaraldehyde)

2. Geodesic sensor net

- Does not allow skin preparation
- Application in about 5 minutes
- High input-impedance amplifiers
- No infection risk



Source: <http://www.brainproducts.com/productdetails.php?id=9>

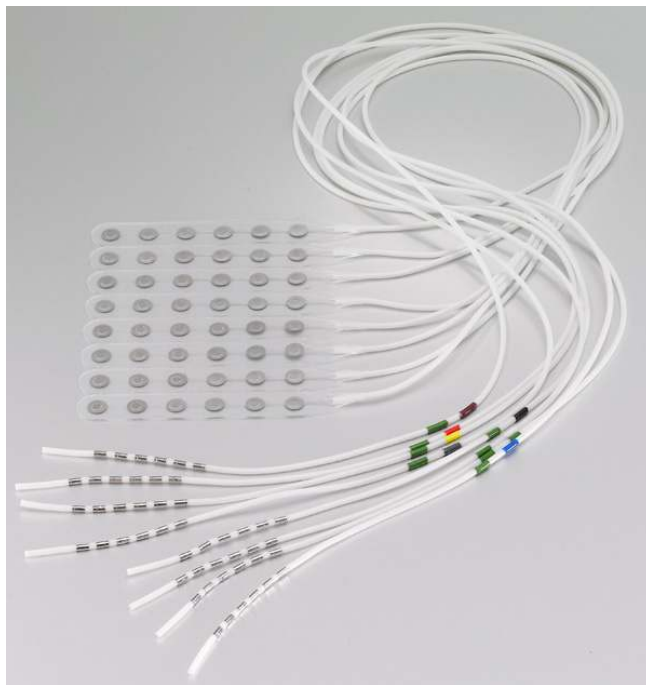


Source: <http://www.miyuki-net.co.jp/jp/seminar/sensornet/sensor03.htm>

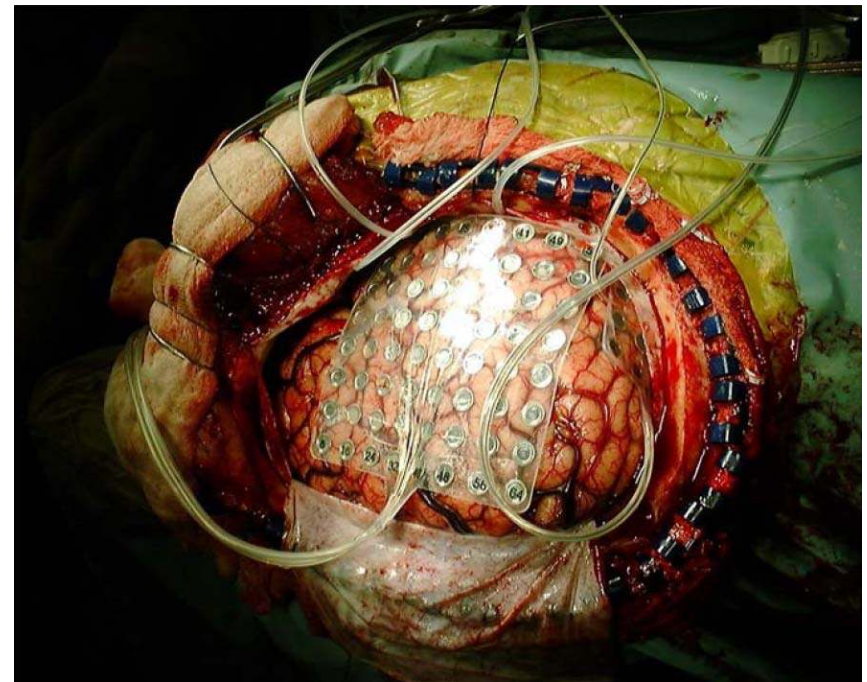
INTRAOPERATIVE ELECTROCORTICOGRAPHY

- Intraoperative monitoring is of high importance: gives valuable information for neurosurgeon during operation about the function of different brain areas
- Electrodes placed not on scalp but right onto cortical surface, this is why these recordings are called electrocorticographical (ECoG) recordings
- Traditionally, this was performed by cotton wick electrodes soaked in sterile saline. The electrodes were placed on the cortical surface and held in place by a metal halo attached to the surgical table
- Modern intraoperative ECoG recordings are performed by subdural grids moved from place to place during surgery

INTRAOPERATIVE ELECTROCORTICOGRAPHY



Source: <http://www.diximedical.net/GB/?cat=27>



Electrode grid for electrocorticography

Electrode grid during brain surgery

RECORDING TECHNIQUES USED IN ANIMAL EXPERIMENTS

- Animal experiments allow researchers to study brain functions in detail and in arbitrary experimental situations
- Both surface (for macropotential recordings) and implanted electrodes (for extracellular recordings) are used
- Implanted electrodes are placed into the brain
- Proper position for implanted electrode determined by brain atlas coordinates
- Stereotaxic technique: electrode placement in a coordinate system, coordinates based on brain atlas
- Stereotaxic apparatus: frame attached around animal's head to keep head stable and measure coordinates relative to markers on head

USE OF STEREOTAXIC BRAIN ATLAS

A brain atlas contains cell and fiber stained brain sections as well as schematic figures based on these sections. Sections are made in all three dimensions:

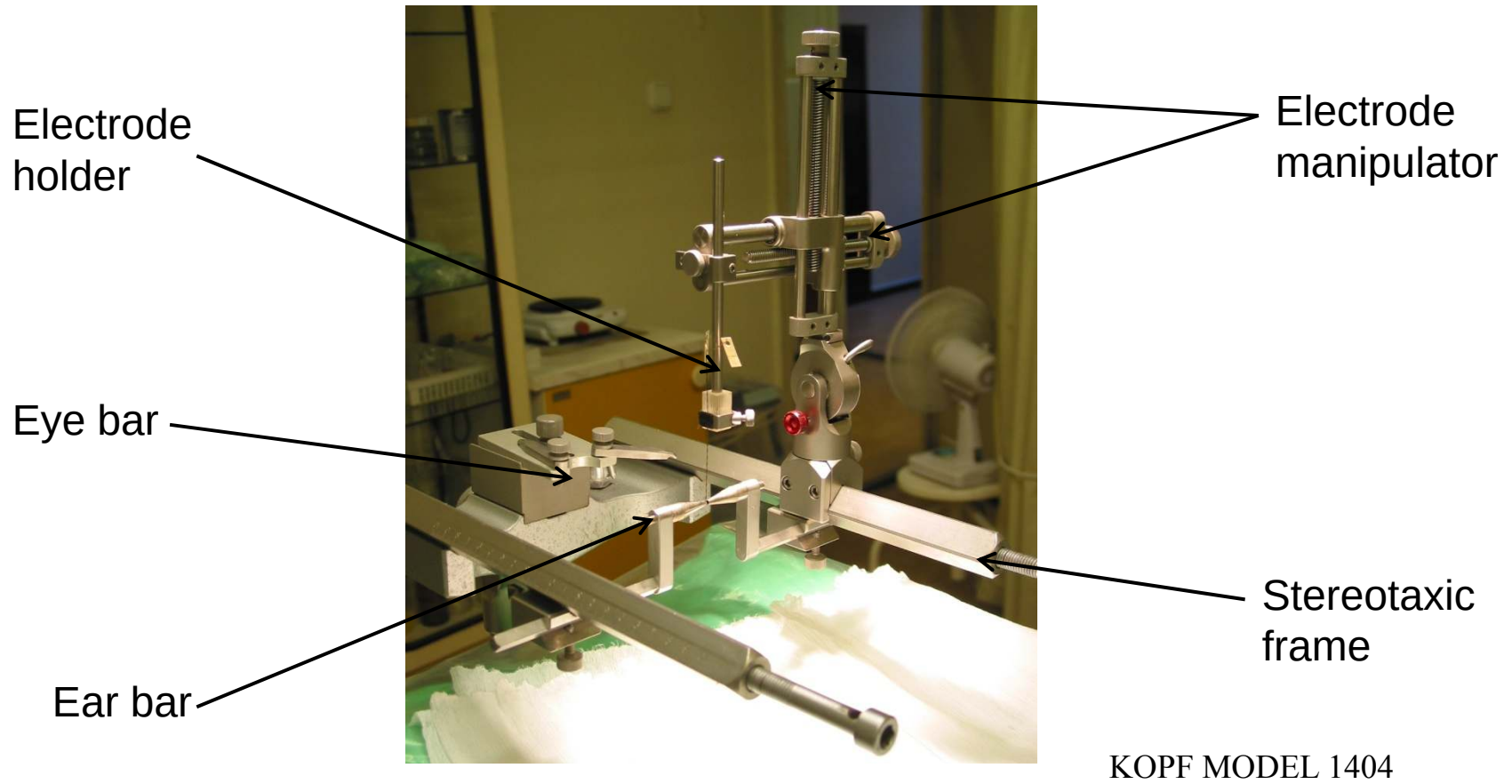
- Coronal: along front-rear (rostral-caudal) axis
- Sagittal: along centre-side (medial-lateral) axis
- Horizontal: along top-down (dorsal-ventral) axis

Coordinates in rats are given according to marker points on the skull: bregma, lambda and interaural axis. Scale bars along the sections show appropriate coordinates for shown structures. With help of a stereotaxic brain atlas an experimenter can precisely determine the desired electrode position.

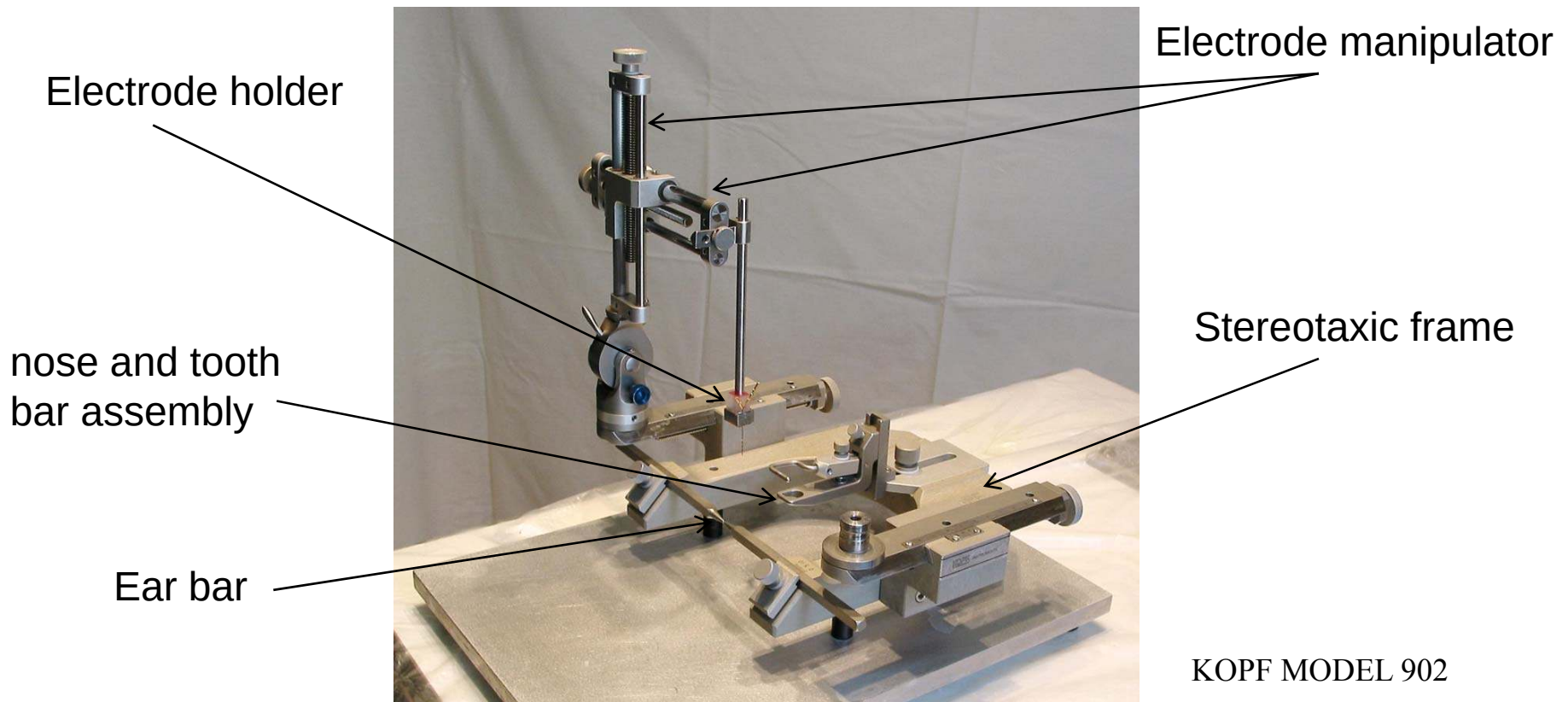
CHRONICALLY IMPLANTED ELECTRODES

- Electrodes implanted during an operation similar to the one seen above are usually implanted chronically. This means that the animal recovers from the operation with the implanted electrodes fixed to its skull. After recovery multiple recording sessions can be performed with this animal.
- The chronically implanted electrode types are usually thin enamel insulated tissue friendly metal wires or multielectrodes. The electrode is insulated in all cases.
- Metal wire electrodes are easy to fabricate, cause relatively little harm to brain tissue and provide stable recordings for long time.
- Multielectrodes have more recording sites, allowing recordings from more brain areas simultaneously.
- However, to maintain stable long time recordings electrode position must be refined periodically. Electrode can move from the desired position either during implantation because of bending of the thin wire in brain tissue or due to brain movement caused by head movement of the freely moving animal.

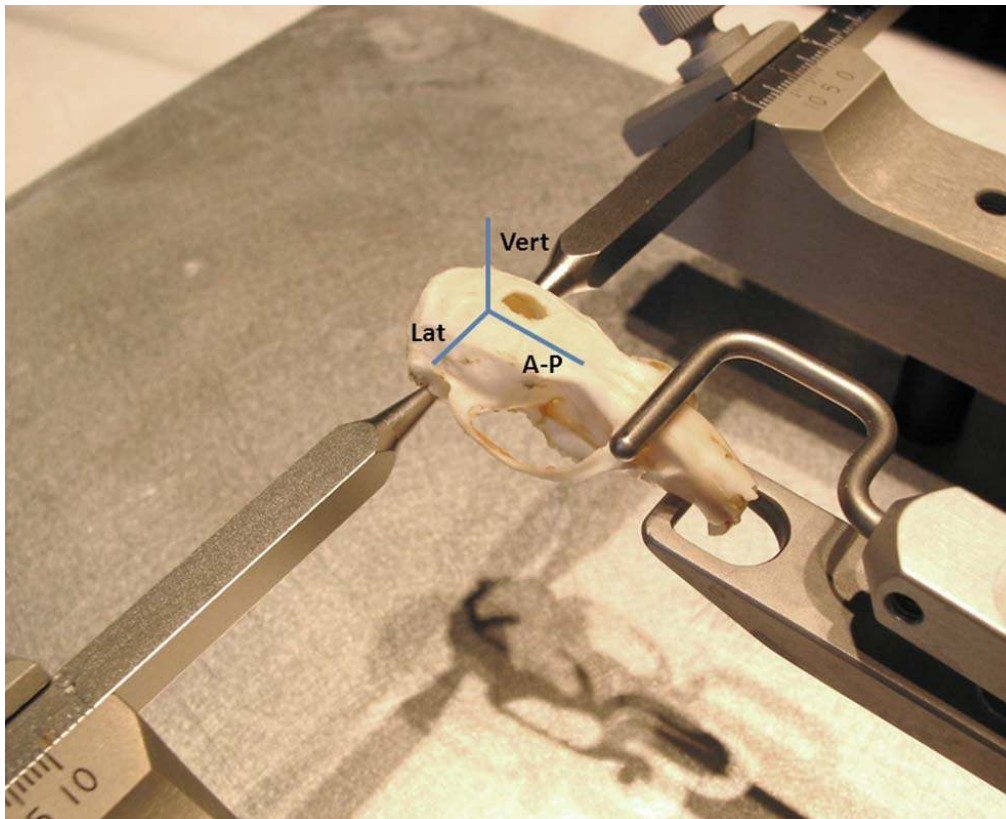
STEREOTAXIC APPARATUS FOR CATS AND MONKEYS



SMALL ANIMAL STEREOTAXIC INSTRUMENT



RAT SKULL IN THE STEREOTAXIC FRAME



The three coordinates are indicated:

A-P.: front - rear direction

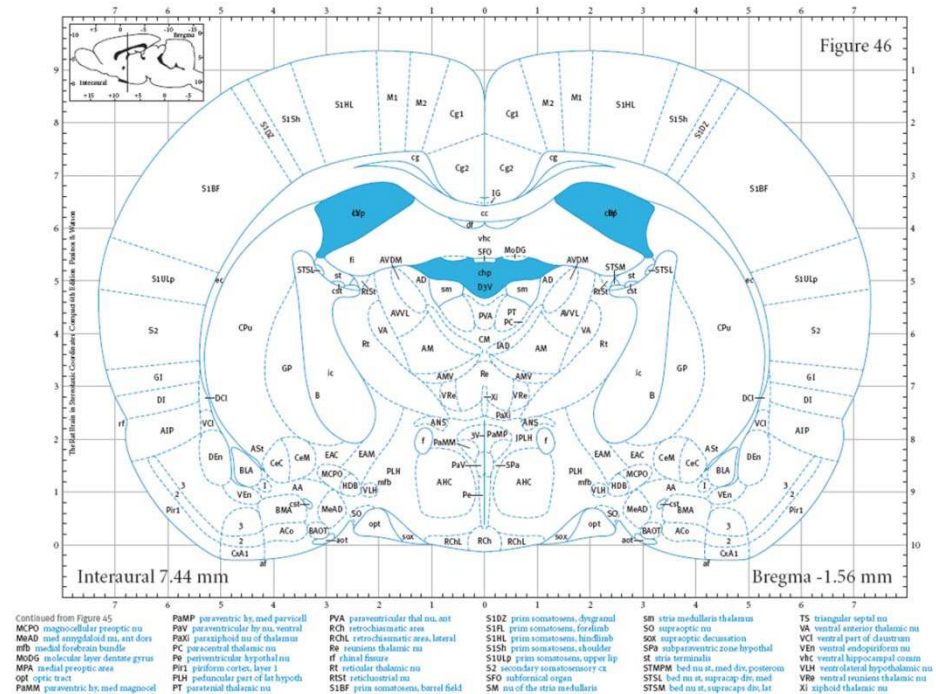
Lat.: medial - lateral direction

Vert.: dorsal - ventral direction

STEREOTAXIC COORDINATES

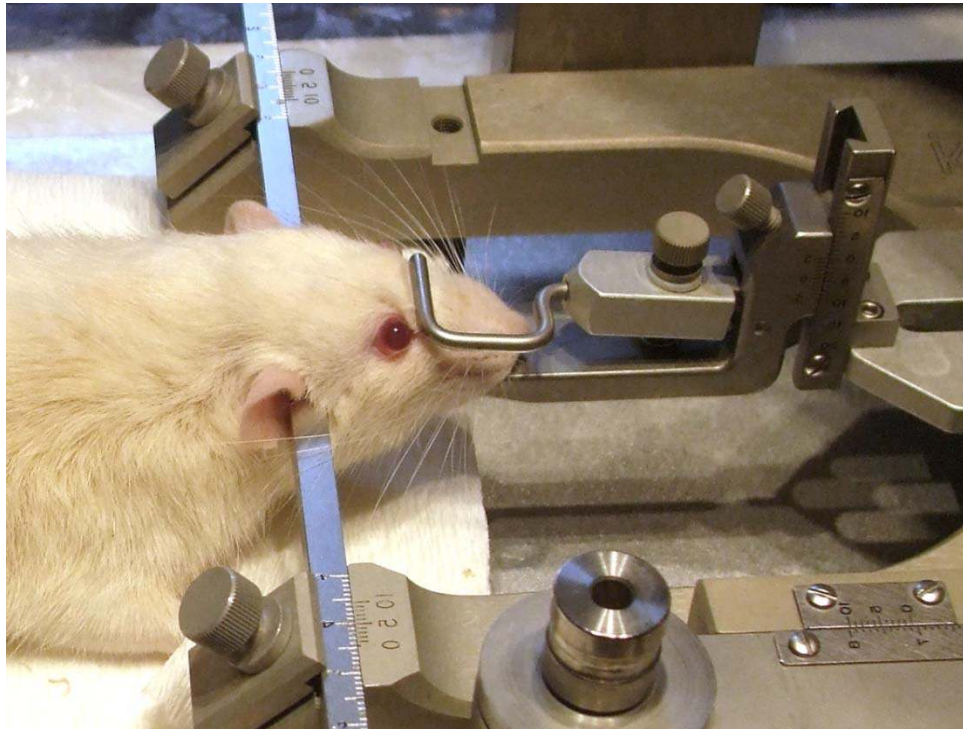


Paxinos, G., Watson, C.,
The Rat Brain in Stereotaxic Coordinates
6th ed., Academic Press, 2009.



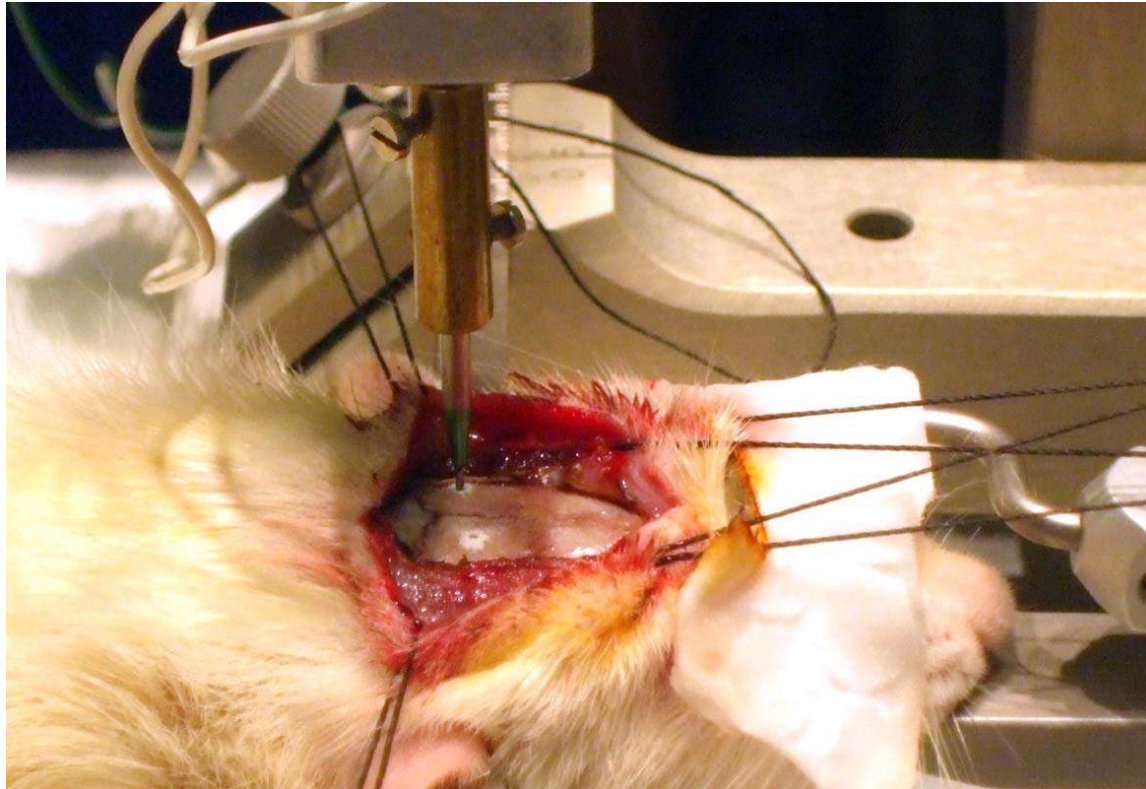
Two pages of the stereotaxic atlas. Left the histological section, right the map of the structures with the coordinates.

ANESTHETIZED RAT FIXED IN THE STEREOTAXIC FRAME



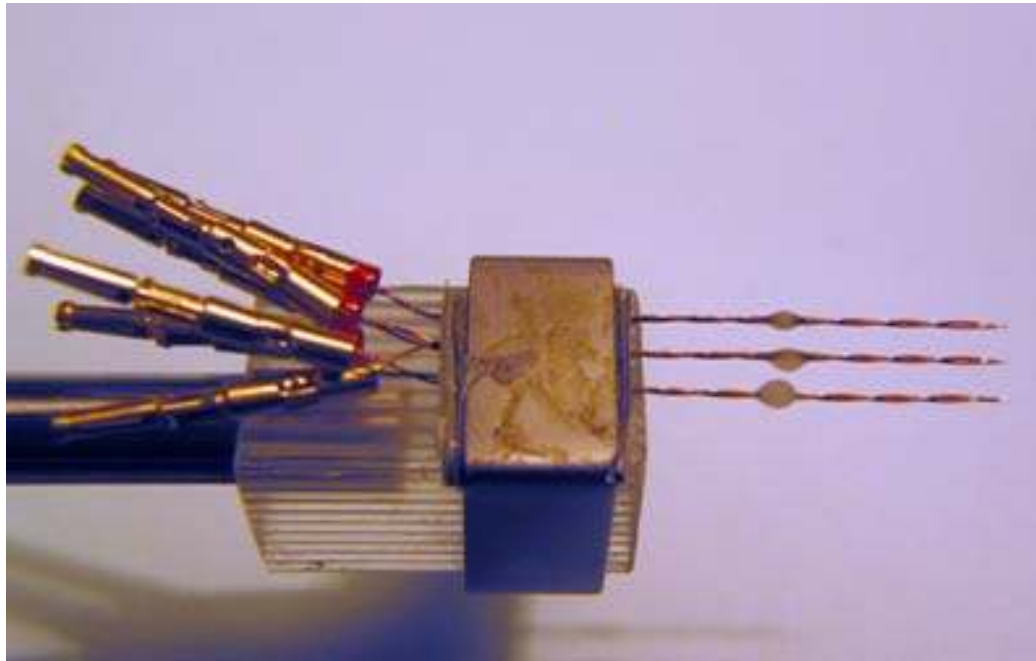
The head is fixed by the ear bars and the nose and tooth bar assembly.

STEREOTAXIC ELECTRODE IMPLANTATION 1



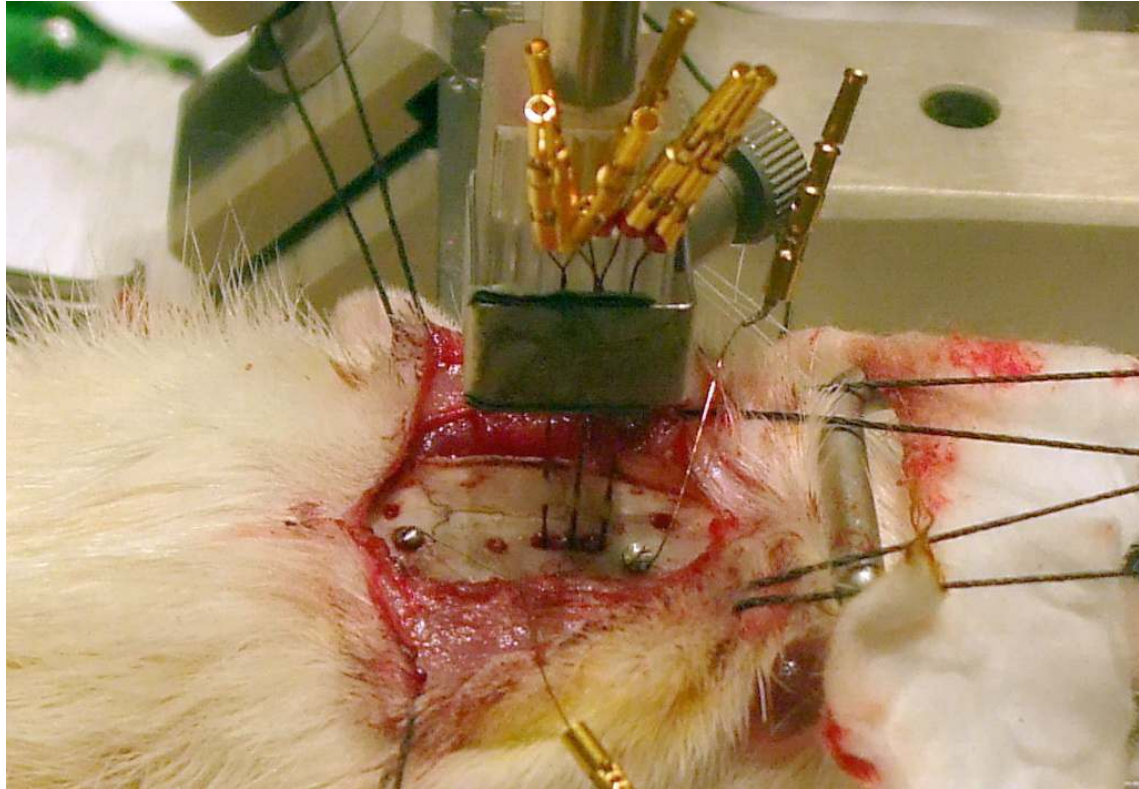
Holes in the skull are made by a drill fixed in the stereotaxic manipulator.

STEREOTAXIC ELECTRODE IMPLANTATION 2



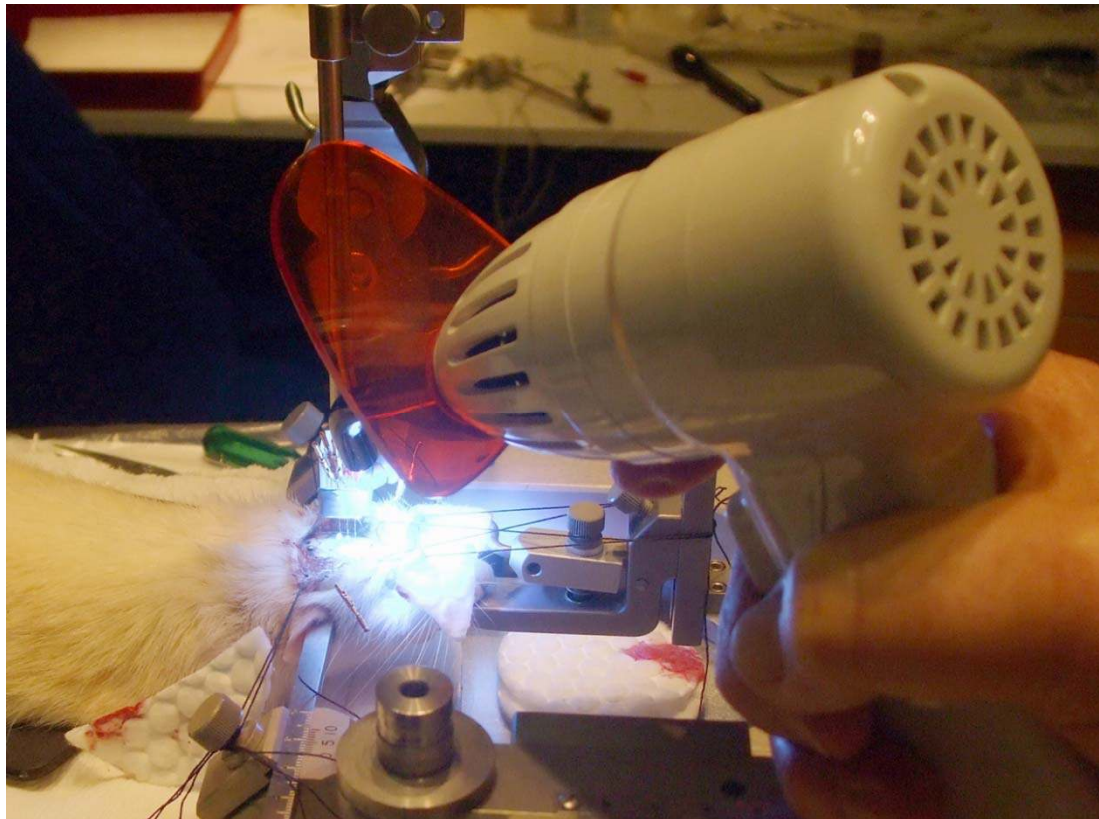
Three pairs of twisted wire electrodes are fixed in the electrode holder. The enamel insulation is removed from the tip of the wires (right). At the other end of the wires female connectors are crimped (left). Drops of adhesive are used to fix the twisted wires.

STEREOTAXIC ELECTRODE IMPLANTATION 3



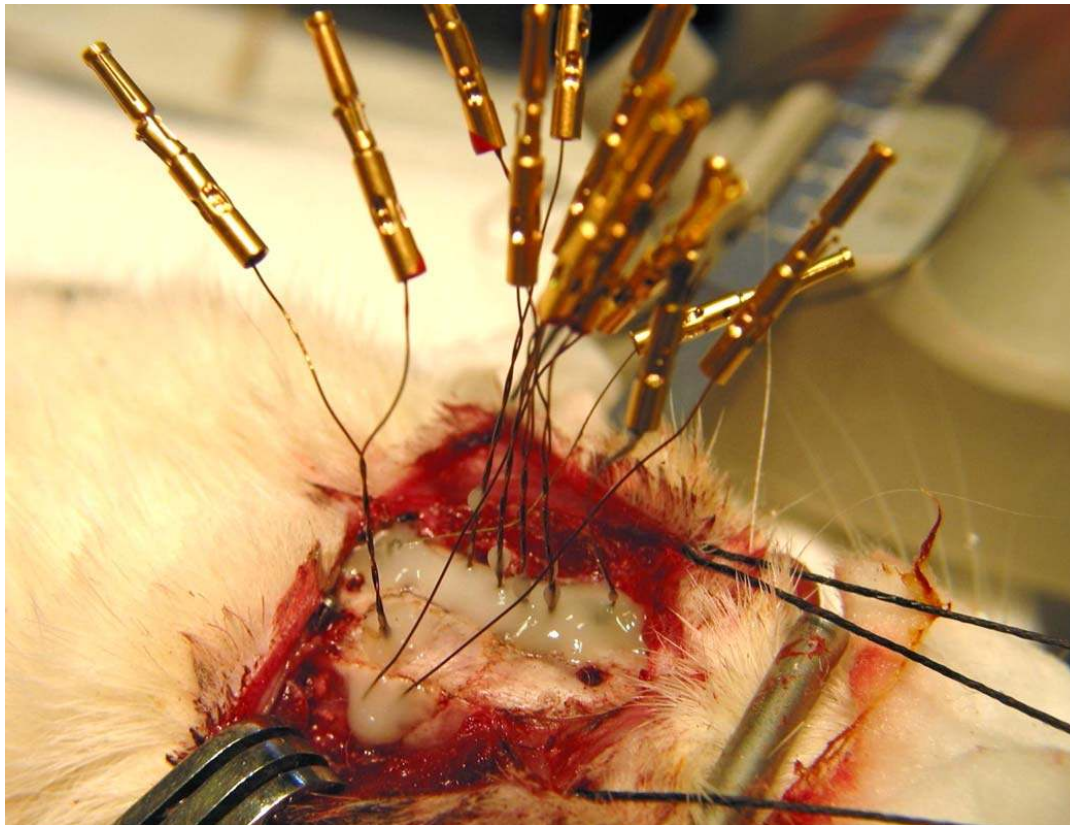
Three pairs of electrodes are inserted into the brain according to the stereotaxic coordinates.

STEREOTAXIC ELECTRODE IMPLANTATION 4



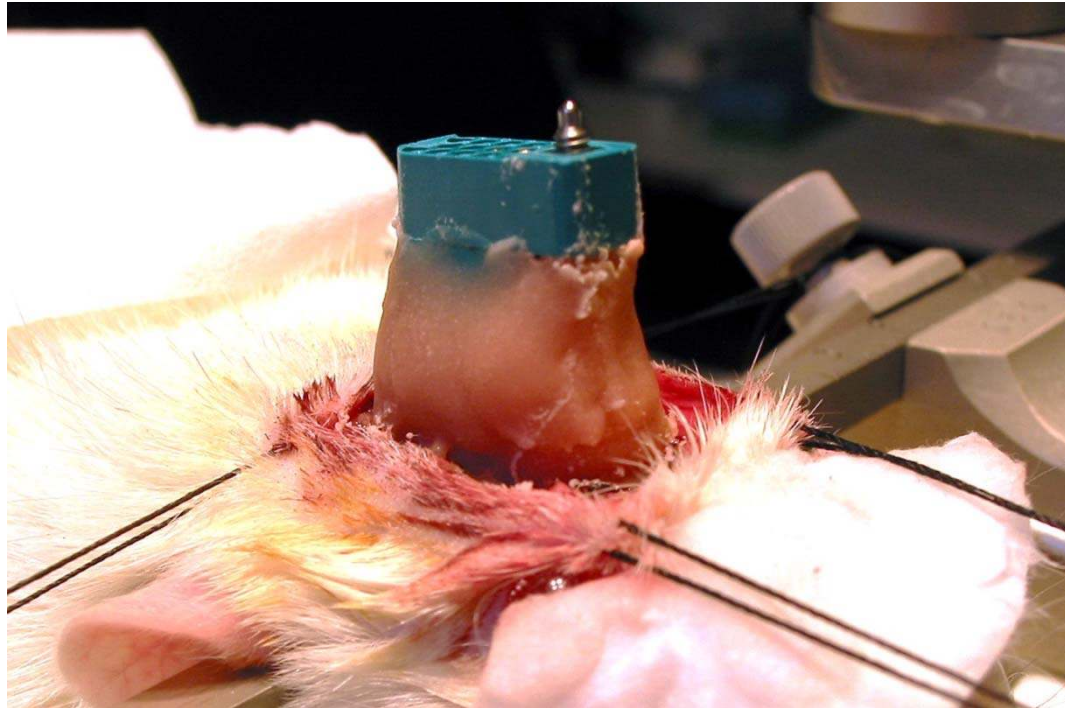
The electrodes are fixed to the skull by light curing dental adhesive.

STEREOTAXIC ELECTRODE IMPLANTATION 5



Seven pairs of electrodes are implanted and fixed to the skull.

STEREOTAXIC ELECTRODE IMPLANTATION 6



The miniature connectors were inserted into the body of the connector and the whole implant are fixed to the skull by dental acrylic. Finally the wound is closed by sutures.

STEREOTAXIC ELECTRODE IMPLANTATION 7



Next day after the implantation the rat is well and ready for the behavioral experiments.

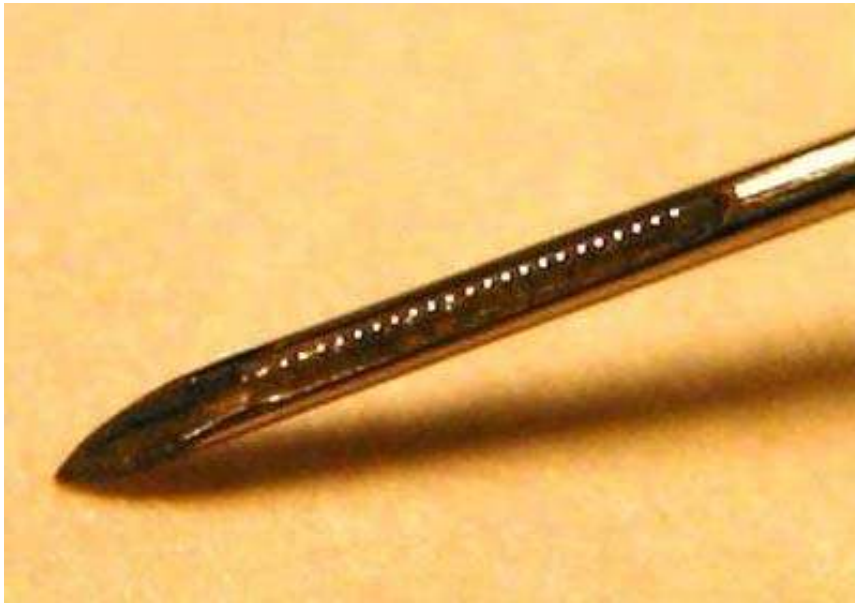
ADJUSTMENT OF CHRONICALLY IMPLANTED ELECTRODES

- In order to adjust electrode position after implantation electrode must be able to move along its axis
- In a simple setup for adjustable electrode a metal wire is inserted into a cannula
- This cannula can slide along its axis with help of a machine screw moved by a microdrive
- The movable cannula is surrounded by a fixed outer cannula for support
- The whole setup is covered by protective housing, with connection cables leading out from the housing to a standard connector
- Electrode position can then be adjusted by fine microdrive movements while monitoring signal quality

TYPES OF CHRONICALLY IMPLANTED MULTIELECTRODES

- Multielectrodes have the great advantage over single microwire electrodes to record from more brain areas at the same time.
- The different types of multielectrodes:
 - Metal (microwire) multielectrode
 - Silicon multielectrode
- Metal multielectrodes are easier to fabricate
- Silicon multielectrode design is pre-defined and highly reproducible
- Microelectrodes and microelectrode recording technique will be discussed in detail in Lecture 6.

TYPES OF CHRONICALLY IMPLANTED MULTIELECTRODES



Metal wire multielectrode



Source: <http://www.neuronexustech.com/catalog2011.pdf>

Silicon multielectrode
(see Lecture 6)

REVIEW QUESTIONS

- What is electrode potential?
- Why does an ideal electrode not exist?
- What is an electrode conductor?
- What is an electrolyte?
- What is the difference between bipolar and unipolar recording?
- Which factors can contribute to signal distortion measured on an electrode?
- How is the double layer at a metal-liquid junction formed?
- Why are non-polarizable electrodes preferable to polarizable electrodes?
- Why is a conductive gel applied on skin at EEG recordings?
- What electrode types are used for biopotential recordings?
- What is electrocorticography?
- What recording techniques are used in animal experiments?

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- www.kopfinstruments.com
- <http://www.neuronexustech.com>