Physiology Practical

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Chapter 1. Rules for laboratory experiments

Physiology is an experimental science gaining its knowledge through observations on living animals, organ preparations and tissue cultures. Observations during medical procedures on human beings also contribute to this knowledge. Experiments done in the laboratories should comply with the existing laws and regulations that are based on bioethical principles. Bioethics is an interdisciplinary science dealing with ethical issues concerning both biological experiments and medical procedures. It is related to the natural and social sciences as well.

1.1. Basic regulations during the practical

1.1.1. Protection of life

The following regulations have to be observed:

- it is forbidden to touch electric equipment or sockets with a wet hand; - it is forbidden to put one's hand inside any equipment;

- a functional error has to be announced immediately to the head of the practical. Cut off the current with the automatic main fuse.

- equipment should be only used as described in the lab notes or explained by the supervisor. Any other operation, turning of knobs, flipping of switches, etc. should be avoided as it might lead to the malfunctioning or impairment of the equipment.

- all the electric sockets are situated on the laboratory benches. Protect them from fluid. Any spillage should be mopped up immediately.

- accidents which occur in spite of all the preventive measures should be reported immediately to the head of the demonstration. He/she is responsible for taking the necessary steps.

1.1.2. Protection of health

Physiological experiments are made on living animals. A non-anaesthetized animal can only be handled by a student in the presence of the technical assistant or head of the practical. Working with living animals implies the risk of infection, as well. Therefore:

- laboratory coats are obligatory

- hands must be washed during breaks or at the end of the experiment. Soap, warm water and disinfectants (Sterogenol and Neomagnol solutions) are available in the laboratory. Use only the designated wash basins.

- eating, drinking and smoking are prohibited in the laboratory area but allowed after thorough hand washing in the lobby of the laboratory.

- before or during the experiment always ask for any necessary first-aid material from the assistants or the experimental supervisor. Suitable disinfectant and antiseptic, finger stalls and hand protection are always available. Working with an untreated open wound may lead to infection and is therefore prohibited.

- in experimental work poisonous and caustic chemicals are used. They should not be put into the mouth or eyes. Certain chemicals (for example, pyridine) and drugs (such as histamine and adrenaline) can be absorbed through the skin: they should be handled with the utmost care.

1.1.3. Other safety regulations

In case of a fire in the building, fire alarm will go on. Elevators will be stopped, and the building should be evacuated through the nearest staircase on foot.

1.2. Rules of experiments on humans

- Human experiments and observation can only be carried out on volunteers after obtaining their informed consent. Subjects should be aware of the circumstances and possible outcomes of the experiment.

- Experiments that might lead to permanent physical or psychological harm of the subjects are prohibited.

- Safety precautions should be entirely followed during the experiments.

1.3. Rules of experiments on animals

Animals perceive external stimuli similarly to human beings, thus they are able to feel pain, and thus possibly they can suffer. Suffering is a subjective term and it is impossible to objectively assess its extent in an animal, but it is generally accepted that vertebrates, especially mammals do suffer. Thus it is the utmost obligation of the experimenter to avoid causing pain to the subject of the experiment, or to minimize it, if it cannot be avoided completely. All experiments carried out during the practical have been scrutinized by the authority dealing with the welfare of animals, and have been approved.

The following points should be taken into account during planning and executing animal experiments:

- Animals to be used in experiments should be kept in an appropriate environment, fed properly, and provided with the necessary social interactions. Sick animals should receive professional veterinary care.

- Researchers and teachers planning and executing the experiments should have appropriate academic background and expertise, and they should be aware the bioethical issues of animal experiments.

- Experiments must have a sound scientific basis.

- Muscle relaxants without general anaesthesia should be only applied if elimination of pain is also ensured.

- Duration of animal experiments should be kept at the minimum.

- Animal experiments are only allowed in institutions that hold the permission of the appropriate authority for that, and only if the knowledge to be acquired in the experiment cannot be obtained in any other way.

- The three Rs of humane animal experimentation should be observed:

  - The number of animals included in the experiments should be reduced as much as possible.

  - Efforts should be made to replace animal experiments using tissue cultures or microbiological preparations.

  - Experiments should be refined, to ensure minimization of pain and discomfort that an unanesthetized animal has to endure without the possibility to avoid it.

- Experiments might be only carried out on animals purchased from an official supplier or bred locally, though in certain circumstances captured individuals of wild animals might be also used. However, to do experiments on stray individuals of pet species (dog, cat) is strictly forbidden.

- Editors of scientific periodicals require a statement from authors that they observed all the laws and regulations concerning animal experiments during their work. Manuscripts describing experiments in which these rules were neglected or violated should be rejected.

1.4. Regulations for the use of the internet

Every workplace in the student lab is equipped with a computer to collect and analyse physiological data and to prepare the report on the experiment. These computers can only be used in accordance of the rules set up by the university and explained by the supervisor.

Chapter 2. Introduction to operative surgery

The main purpose of the labs in physiology is to study the physiology of organs and organ systems and the organism as a whole under normal and experimental conditions. Experiments are always performed on living animals or isolated, surviving organs. In both cases adequate physiological conditions should be provided. Visual observation is complemented and helped by recording and measuring with instruments. Results, observations and conclusions should be recorded in your notebooks.

2.1. Anaesthesia and pain relief

Several methods are available to prevent pain perception during an operation. Local anaesthesia means the reversible blockage of synaptic transmission or nerve conduction in the surgical area. In this way the pain impulses are not transmitted to higher centers in the central nervous system. General anaesthesia means the overall blockage of synaptic transmission in the nervous system. Adequate anaesthesia implies the prevention of pain perception without blocking other physiological functions. These criteria can be met by using agents with a broad spectrum of actions. The anaesthesia levels reached by currently used narcotics are, in ascending order, the cortex, the subcortical structures, the cerebellum, the medulla and the spinal cord.

The effects of drugs can be regulated by careful selection of the dose. Dosages of anaesthetics per body weight for the most frequently used laboratory animals are available in cumulative tables. The tables also show the range of effectiveness: this is the difference between the median effective dose, ED_{50} and the median lethal dose, LD_{50}. (The ED_{50} is a dose that produces its effect in 50 per cent of a population, while the LD_{50} is a dose that is lethal for 50 per cent of the test subjects.) The larger is this range, the less harmful is the use of the given drug. Under adequate anaesthesia, the circulatoryand respiratory functions and muscular tone are preserved (for the release of the latter-, muscle relaxant is given). Most of the complications during anaesthesia are related to respiration. It is also essential to know that body temperature decreases during anaesthesia. Therefore, animals undergoing a long operation should be kept warm. Most anaesthetics are fat-soluble: they exert their effect by inducing structural changes in the cell membrane of nerve cells.

General anaesthetics can be classified as follows:

- volatile liquids (ether, chloroform, halothane)

- gases (nitrous oxide, ethylene, cyclopropane)

- intravenous aesthetic drugs, solutions (barbiturates, pregnanedion, ketamine and urethane)

In current surgical practice volatile or gaseous anaesthetics are used. In animal experiments, however, solutions are preferred, because of their simplicity. They may be administered either intraperitoneally (i.p.) or intravenously (i.v.). The first method is easier but the latter is more effective and allows more precise administration of the drug. Depending upon the dose and time of anaesthesia, different depths of anaesthesia are distinguished:

- analgesic period; - no perception of pain, decreased reflex threshold;

- excitatory period; - extinction of cortical inhibition, lack of consciousness, appearance of unconscious escaping reactions, uttering sounds, increased vegetative functions;

- period of tolerance; preferred state for surgical interventions; - subcortical motor centers are blocked, reduced tone, extinction of somatic reflexes indicated by the lack of the cornea reflex

- period of respiratory arrest; no activity in the medulla

In physiological demonstrations different anaesthetics are used. Short operations might be carried out under ether anaesthesia (inhalation technique). In the case of longer operations, ketamine + xylazine, Nembutal (sodium

pentobarbital) or urethane (ethyl carbamate) is used. Urethane is only used in operations after which the animal is sacrificed.

Urethane is a potent aesthetic for a long experiment, and it affects a wide range of neurotransmissions. Chloralose, often in the form of urethane-chloralose cocktail, is used in some neurophysiological experiments. It does not affect reflex and cerebral electric activity but its administration is rather complicated and requires a certain degree of skill.

Blockage of nerve conduction is usually produced by Procaine or Lidocaine local anaesthetics.

2.2. Immobilization of the animal

Following general anaesthesia, or decapitation (frog), the animals should be fastened to the operating board, in order to perform the interventions in a precise and safe manner. The actual method for fixing their body depends on the species and the type of operation.

In the case of frogs, sufficient immobilization can be reached by piercing the extremities to the wax bottom of the frog dish. With rats, however, the limbs should be fastened with a thread to the operating board. It is also advisable to keep the animal's head in position using the upper incisors. When working with non-anaesthetized (alert) rats (e.g., while taking blood samples from the tail vein) the animal is placed in a cone, approximately 5 cm in diameter, that makes only its tail accessible. Alert rabbits can be examined in stocks. Among laboratory animals rabbits are the most tolerant to medical interventions and the least sensitive to pain. Before surgery to be performed under anaesthesia, the fore-limbs of the rabbits have to be tied crosswise under their back and the hind-limbs stretched apart. The position of the head should also be fixed with a thread threaded on the upper incisors.

For the study of the brain, the head is fixed in a precise framework using a stereotaxic apparatus. For a description and instructions for use of the stereotaxic apparatus see the corresponding chapter.

2.3. Injections

In physiological experiments parenteral administration of drugs, solutions, etc. may often be necessary. In such cases the agents should be injected. Types of injection are:

Subcutaneous (s.c.): lift the skin slightly and pierce it in one go. Check that no tissue damage has been caused and inject the fluid. If there is a large volume of fluid, pull back the needle slightly to prevent all the fluid from accumulating in this area. In frogs there are lymph-sacs under the skin. The injected fluid is readily absorbed from them. The lymph sacs are separated from one another by septa.

Intramuscular (i.m.) injection: with this route of administration drug absorption is faster and at a more even pace. Injections are also less painful. Injections should be made quickly with a firm movement.

Intraperitoneal (i.p.) injection: The abdominal cavity is the preferred site for injections for small laboratory animals in which other routes of administration are hardly feasible. Injection means piercing the peritoneum. Take care not to injure the internal organs (liver injury with lasting and profuse bleeding may be particularly dangerous). Intraperitoneally administered drugs are rapidly absorbed but their effect is strongly modified by the amount of chyme in the gastrointestinal tract. Doses listed in the table relate to animals starved for 24 hours before i.p. injection; drug effects cannot be calculated in animals not starved before the experiment.

Intravenous (i.v.) injection: it makes fast and precise administration possible. Its disadvantage lies in the difficulty of finding an adequate vein. In the case of rats and mice the tail vein is used. It can be slightly dilated in warm water. An effective result can only be achieved with a thin needle. In the rabbit the ear vein is easily localized and it can be dilated sufficiently with a cotton swab soaked in xylene. During this procedure the rabbit is placed into stocks. Injection of cats and dogs requires detailed knowledge of the various techniques. With tame animals (particularly dogs), however, it can be performed in an alert state, too. Otherwise it is advised to apply a short-term pre-anaesthesia e.g., with ether. When making an intravenous injection, be careful to insert the needle parallel with the vein to prevent its passing through the vessel wall. Compression of the proximal end of the vein leads to the accumulation of blood at the injection site that makes it much easier to keep the needle level. If surgery necessitates repeated intravenous injections it is a good idea to insert a venous cannula in pre-anaesthesia.

2.4. Preparation of the area of the operation

The animal's fur disturbs surgical work: it contaminates the area of operation and in chronic operations it may become the source of infection. Consequently, it should be shaved off before surgery. In the case of rats or mice the hairs can be plucked out by hand or by forceps (they regrow within a few weeks after chronic operations). With larger animals (rabbit, cat or dog), the fur can be shaved with scissors (Cooper's scissors): take care not to injure the skin. The cut hairs can be removed with a wet cotton swab. A simpler solution is to use a small animal clipper with appropriate cutting head that leaves very short hairs only. Before chronic operations the area to be operated on should be painted with iodine or alcohol. This is not necessary for acute experiments.

2.5. Types of operation

In physiological demonstrations we frequently make acute interventions, i.e. at the end of the experiment the animals are killed. There are other experiments, however, when chronic operations are needed. This means that animals come out of anaesthesia and are let to recover. The most important types of operation used in physiological experiments are as follows:

In situ preparation:

This means the observation of an organ and its functioning without disconnecting it from its vicinity. The organ itself is also not exposed but it is liberated from the surrounding connective tissue. For instance, the "in situ frog heart" preparation is like this.

Isolated organ

The organ to be examined is removed from its original site and put in a container. It functions satisfactorily in the artificial medium of the organ bath. Examples of isolated organ preparations are "Straub's heart preparation" and "The Magnus-type isolated intestinal segment".

Extirpation or destruction (lesion)

An organ or a part of it is completely removed or destroyed. Functional changes caused by the absence or damage of the given organ are studied. Functions of the endocrine glands and certain cerebral regions can be studied by this technique.

Cannulation

By the insertion of cannulas in organ cavities agents can be injected in or fluids drained out. For example, the cannula inserted into the trachea ensures that the anaesthetized animals can breathe without difficulty, while an arterial cannula can be used to measure blood pressure changes. Similarly, a venous cannula can be used to administer fluids into the body, and gastric and intestinal cannulas can be used to obtain samples of digestive fluids. Urine samples can be obtained using a ureter cannula.

Implantation of electrodes

Electric stimulators (electrodes) are placed primarily on nerves. The nervus vagus is stimulated to study its functions in the heart, lung and stomach activity, while the nervus ischiadicus is stimulated to study sensory and motor activities. Recordings are made using electrodes connected to a suitable recording apparatus.

Naturally, the basic types of operation can be applied in combination, as well. A complex physiological experiment might require cannulation, implantation of electrodes and organ isolation.

2.6. Physiological requirements

In order to investigate animal physiology, proper physiological conditions should be provided during the experiments. Three conditions are of particular importance:

- composition of agents administered and the milieu of the isolated organ (organ bath),

- adequate temperature,

- respiration, cardiac activity and blood pressure of the animals.

Fluids injected into the body or used for the perfusion of isolated organs are based on a substance known as "physiological saline". From the point of view of osmotic pressure, ion composition or pH value they resemble the body fluids which they replace. Their actual composition may vary depending on the purpose of the experiment (see Appendix).

In addition to the pH value, the temperature of the injected fluids should be adjusted. Normal body temperature is preferred, particularly in the case of i.v. administration. The body temperature of anesthetized animals must also be controlled. The body temperature of smaller animals can be maintained at a satisfactory level with an electric pad or a hot water bottle (warming is particularly necessary in the "Complex Physiological" and "Sensory Evoked Potential" experiments). Surgically exposed body cavities should always be warmed, since internal organs are more sensitive to a fall in temperature. If this is not feasible, a cotton swab soaked in warm physiological saline should be put over the open wound.

Respiratory failure may be due to anaesthesia. It may be aggravated if saliva gets into the trachea of the animal lying on its back or if the tongue bends back and obstructs the pharynx: these complications can be prevented by pre-operative cannulation of the trachea. If it is not feasible for any reason, administer atropine-sulphate to reduce the intensity of salivation.

2.7. Surgical instruments

The most important surgical instruments used in physiological experiments are as follows:

Cutting tools

Skin is usually incised with a scalpel (surgical knife) or occasionally with surgical scissors (Figure. 1b). Fur is shaved off with Cooper's scissors with bent blades. To avoid injuries of the internal organs, when cutting tissues under the skin, it is advisable to use scissors, which have one blade blunt. Bony areas are cut with bone cutting forceps (Liston's forceps). The skull can be exposed with a trephine (a crown saw for removing a circular disk of bone). A raspatory (xyster) is a file or rasp used to remove membranes from the bone surface. Internal organs (vessels, nerves, tissues) might be incised or cut by ophthalmic iris scissors. It is always essential to use sharp tools.

Forceps

Different types are in use. Hooked tongs are used for seizing skin portions. Other tissues are lifted up by smooth (dissecting) forceps. In experiments with internal organs dental or ophthalmic forceps are used. The latter have both hooked and smooth pointed versions. Some experiments might need the use of artery forceps by which circulation to organs or tissues can be blocked. Artery forceps can be locked by the serrated cross plate near the fingers so they can be left in place without having to hold them. The smooth pointed versions are called Pean forceps and the hooked ones Kocher forceps. The so-called "mosquito forceps" are very small artery forceps while the "bulldog" forceps are larger.

Instruments for closing wounds

There are two methods of closing a wound, suturing and stapling. Suturing needs a needle holder and surgical needles. The needles may be sharp with a triangular-shaped cross section or serous ones with a circular cross section. They are classified and numbered by their diameter, length and type. Low numbers indicate small diameters. The ending of the needle allows for the thread to be simply forced into the hole as it is not a closed ring. In recent surgical practice the so called pre-threaded "atraumatic needles" with a smooth surface dominate. In these needles, the needle and the thread are integrated during fabrication that also means that one needle can be used only to make one stich. Most of the threads are made of sewing silk; however, they can also be made of cotton, synthetic or organic material. Threads and prepared in different thickness. The internal organs are usually sutured with absorbable, organic catgut. In other cases, the thread should be removed following complete healing to prevent in-

flammation. Staples are usually applied to close skin incisions: after the wound has healed they are removed. A special instrument is needed to insert and remove them.

Figure 2.1. The most often used surgical instruments. Upper row: pointed metal rod for destruction of spinal cord in frogs, scalpel (lancet or surgical knife), scalpel with replaceable blades, surgical scissors, ophthalmic iris scissors, smooth (dissecting) forceps and hooked tongs, dental forceps, ophthalmic forceps, artery forceps (hooked, i.e. Kocher), galvanic forceps (made of copper and zinc). Lower row: Pasteur pipette, spreading retractor, bone cutting forceps (Liston's forceps), needle holder with different surgical needles, clip (staple) applier-remover with staples, artery forceps (smooth, i.e. Pean)

Other instruments and tools

Liquids are administered with injection syringes and needles or cannulas. Two syringe standards developed in parallel, differing in the diameter and angle of the cone of the syringe: Record (Europe) and Luer (USA). The latter one also has a simple needle-lock system to fix the needle on the syringe that is very important when pressure is needed to inject a liquid. By now, the Luer standard practically made the Record standard obsolete and replaced it in almost every application. Capacity of syringes has a wide range from 1 ml (called Tuberculin syringe) to 2, 5, 10 or 20 ml. Organs can be perfused with a larger volume of fluid using even larger syringes with a capacity of 50-250 ml. Syringes are made of heat-proof glass or more recently of plastic.

Cannulas to be inserted in organ cavities are made of glass or plastics (polyethylene, silicon, Teflon) in different sizes. The glass cannulas are mouldable, so bulbs and hooks can be made in them (Figure. 4.b). Tracheal cannulas are "T" or "Y" shaped and the arterial ones have branches at the side. Straub's cannula has a special shape with a pointed orifice and a hook. Organs are moistened by spraying them with a Pasteur-pipette: the fluid can be directed to the required area by moving the rubber hose of the pipette.

2.8. General design of experimental surgery

Preparation - selection of the animal, anaesthesia, fixation of the animal's position on the operating board, cleaning the area of the operation.

Preparation of the surgical instruments

Surgical exposure - the skin is incised with a scalpel or scissors. Muscles bundles are usually separated with blunt dissection to avoid excessive bleeding, except the abdominal wall where one can use scissors or scalpel along the tendinous linea alba. The skull is exposed with a trephine and bone forceps.

Hemostasis - cutting induced bleedings can be mitigated or arrested in many ways according to their nature, e.g.:

• capillary bleedings are mopped up;

- vessels are temporarily clamped with arterial forceps (Pean's, Kocher's and mosquito orceps) for prompt hemostasis;

- ligation, when chronic bleeding can only be terminated by a thread of silk threaded under the bleeding vessel. (Be careful not to involve other tissue.);

- use of auxiliary materials, like "fibrostan" which is a sponge of fibrin-like material to promote coagulation;

- bone wax, which is an aseptic surgical wax, a boiled mixture of beeswax and paraffin applied to the cut surface of the bone to stop bleeding;

- cauterization (coagulation of tissue proteins by high frequency alternate current flowing between the small surface of the cautery and a large reference electrode) is often used in major operations.

In certain experiments, however, the very purpose is to prevent coagulation. Blood samples or blood that has got into the cannula of the tonometer can be prevented from coagulating, by using heparin, which is a physiological anticoagulant or by citrate able to bind the Ca^2+ ions.

2.9. Preparation

The anatomical region to be studied is exposed, i.e. the adjacent tissues are detached or removed, if necessary. The major vessels of the organ to be removed are excised between two ligatures (e.g., the heart). Vessels selected for cannulation and nerves to be used for stimulation or recording should be stripped of connective tissue, and if necessary, the prepared area should be held with a thread for any further manipulation (e.g., ligation, lifting up, etc.) Cannulas or electrodes are likewise fixed with a suture. Take care that the given organ is not twisted or broken. If more than one instrument is to be placed in the same animal one has to plan in advance the order in which they will be put in, so that the instruments put in first will not interfere with those put in later. It is a good idea to leave the most complicated operations until last, e.g., cannulation of the common carotid artery. If necessary, the cannula or electrode can be fixed to the adjacent connective tissue with a suture.

2.10. Wound closure: sutures and staples

Acute operations may also require wound closure: for example, in order to maintain body temperature. Muscles and viscera should be sewn with a continuous suture using a cylindrical needle; while skin wounds should be sewn using interrupted suturing. The latter will hold even if one or two sutures are rejected or torn out by the animal. Sutures should always be made with double or surgical knots. Internal organs should be sewn with suture no.0, the muscles with suture no.1 and the skin with a somewhat thicker suture; ligation and cannulations are also made with thicker sutures. Stitching is easier if the given tissue is lifted up with forceps.

2.11. Postoperative treatment

After chronic operations the animals are covered and taken to a warm room until they wake up. Antibiotics or other drugs have to be administered according to need. On the first post-operative day the animals should be given light food. than they can be gradually re-accustomed to normal food. After the completion of the operation gather and remove the debris. At the end of acute operations animals should be killed with an overdose of an aesthetic and disposed according the regulations. Surgical instruments, cannulas, dishes and benches should be washed and returned to their place. The practical is only over when the working place and the surroundings have been cleaned and all the electric equipment has been switched off.

Chapter 3. Biopac Student Lab (BSL) System

The Biopac Student Lab System is an integrated, computer controlled data acquisition and analysis system for non-invasive human physiological studies.

3.1. Parts of the system (MP30/35/36)

Figure 3.1. MP30 data acquisition unit. The device is connected to the computer by USB cable.

The measuring system is based on the MP30/35/36 multi-channel amplifier/filter unit, which is suitable to receive and process signals from 4 equivalent inputs referred to as channels. The input ports are located on the front of the MP3x units and are labelled as CH1, CH2, CH3, and CH4 (Figure. 3.1). During measurements, electrodes and transducers are connected to the inputs, thus changes in electrical potentials on the body surface (in EMG, ECG, EEG, EOG measurements) or different biological variables and their changes (pressure, volume, etc.) can be monitored and recorded by the system. Data collected from the input ports are displayed by the acquisition program (Biopac Student Lab) under the appropriate channel number.

On the front, the „POWER" led shows the status of the device, while the „BUSY" led indicates whether data collection process or internal calibration of the device is going on.

Before you set up an acquisition process, make sure that the BIOPAC MP3x unit is in OFF state! Never connect electrodes or transducers to the acquisition unit while it is the ON state!!

3.2. Data acquisition with the BSL program

During the practical, Biopac Student Lab (BSL) 3.7.6 program is used for data acquisition and analysis. The presets of the different measurements are called "Lessons" in the program. Lessons are set up so that you can record data in the lab and then analyse the data later (in the lab or at home). To run a BSL Lesson, the MP3x unit should be in "ON" state and connected to the computer.

After starting the program, the appropriate lesson should be selected from the "Lessons" drop down menu and the person(s) who is performing the test should be identified. Each lesson starts with *calibration*, when the system automatically optimises the amplification of the signal. After a successful calibration, *measurement period* is started. During measurement, data collection can be interrupted and restarted at any time. Each recorded data set is automatically saved at the end of each lesson recording. It is not necessary to save the data manually. Saved data can be retrieved at any time by its identifier. Generally, the following control buttons are available during the measurement(s) (individual lessons might slightly differ from this):

Calibration period		Measurement period	
Calibrate	Automatic signal optimization by setting up the gain	Record	Start recording
Redo calibration	Repeat the calibration and erase the old data	Suspend	Pause recording
		Resume	Continue recording
		Stop	Finish data acquisition
		Redo	Repeat the last recorded section and erase the old data
Done	Finish the lesson and start the analysis		
F9	By pressing *F9* function key, event markers can be inserted into the recording. Using the markers, you can identify the time points of your interventions.		

You can analyse your measured data with Biopac Student Lab (BSL) 3.7.6 in the lab or with BSL Analysis 3.7 at home, which is free to download from the Department's web site. For the installation, unzip the file, then run the *.msi* installer and follow the instructions on the screen.

3.3. Data analysis

Data analysis is carried out on the BLS display, which includes a Data window and a Journal. The Data window displays the waveforms and it is the place, where you will perform your measurements and analysis. The Journal is where you will make notes. You can extract information from the Data window and put it in the Journal and you can export the Journal to other programs for further analysis. Your data file contains both the collected data and the Journal so you can stop the analysis at any time and continue it later.
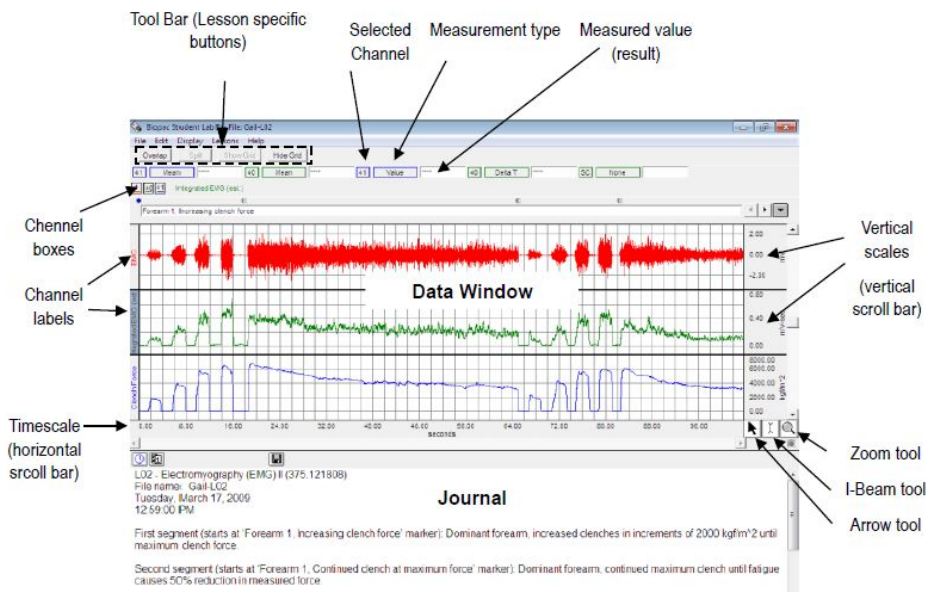
Figure 3.2. *Overview of the BLS display and the controls.*

On the program's user interface, there are three main control tools to edit and select your recorded data:

⇱	**Arrow tool**: mouse works in the regular way
🔍	**Zoom tool**: enlarge the selected area
I	**I-Beam tool**: select a segment of the record; the selected segment is displayed with inverted colour

Measurements are performed in the Data window, where the measurement tools are used to extract specific information from the recorded waveform(s). At the beginning, you should choose the **Arrow tool**, and scroll to the section of the recording to be analyzed by using the horizontal scroll bar. If the amplitude of the waveform(s) is not appropriate (to large or to small), the „*autoscale waveforms*" option from the **[Display]** menu is a quick way to fit the waveforms to the channel's window. „*Autoscale waveforms*" sets the *vertical scale* of each channel window, so the waveform fills approximately two-thirds of the available area. „*Autoscale horizontal*" option is useful to fit the entire waveform within the data window. (It will adjust the horizontal scale in such way that the leftmost portion of the screen will be the beginning, while the rightmost portion the end of the recording.) To expand selected sections, you can use the **Zoom tool**. (The selected segment is expanded to the available area, in order to show more details.)
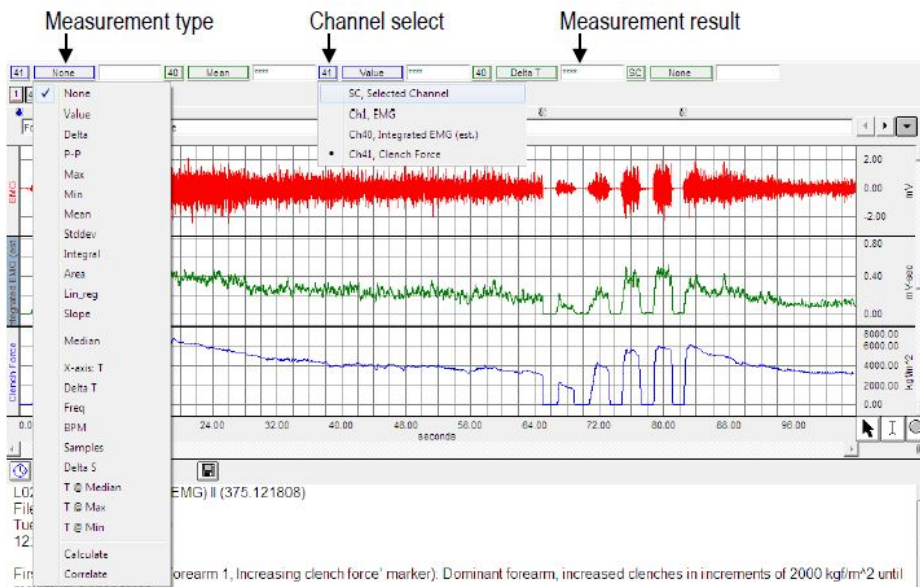
Figure 3.3. Frequently used measurement tools for data analysis

To use the measurement tools, you should select an area for measurement by using the **I-Beam tool**. The selected area is displayed on the screen with inverted colour. The correct selection is absolutely important, because the measurement only applies to data in the selected area of the waveform that the user specifies. Please, note that the program is not able to automatically recognize the frequency of the repetitive waveforms, such as R waves in ECG recordings; "freq" simply means the reciprocal of the "length" of the selection. Once the area selection is done, select the channel to be analysed (C*hannel select*). By clicking on the *measurement type* button, select the desired measurement type from the pop-up menu. The value for the selected parameter (type) will appear immediately in the *Measurement result* area (Figure. 3.3).

The most frequently used measurement types are:

The displayed values in the *Measurement result* areas can be copied and pasted to the Journal by pressing the **Ctrl+M** keystroke command. The Journal is a simple word processor, so you can type notes or copy measurements from previously saved data. (The Journal needs to be the active window for its options to come up.) If you want, you can export the text/data from the journal to another program by choosing the **Copy** command from the **[Edit]** menu.

Copying the content of the Data window is also possible. Choosing the **Data window/Copy graph** option from the **[Edit]** menu, you can copy the waveform data as a picture to be imported into other programs.

You can print your data with the **Print** option of the **[File]** menu. In this case, you will be prompted to choose which items to print. „**Print graph**" will print the selected area or the content of the Data window; „**Print journal**" will print the content of the journal.

value	The value measurement displays the amplitude value for the channel at the point selected by the I-beam cursor. If an area is selected, the value is the endpoint of the selected area.
delta	The Δ (delta amplitude) measurement shows the amplitude difference between the last and the first point of the selected area.
p-p	The p-p (peak-to-peak) measurement shows the difference between the maximum and the minimum amplitude value in the selected area.
max	The maximum measurement finds the maximum amplitude value within the selected area (including the endpoints).
min	The minimum measurement finds the minimum amplitude value within the selected area (including the endpoints).
mean	The mean measurement computes and displays the average amplitude value of the data samples between the endpoints of the selected area.
delta T	The ΔT (delta time) measurement is the difference in time between the end and the beginning of the selected area.
freq	The frequency measurement converts the length in time of the selected area to frequency in cycles/sec by taking the reciprocal value.
BPM	The beats per minute (BPM) measurement uses the length of the selected area in time as a measurement for one beat, and based on this time, calculates the approximate BPM value.

Chapter 4. Analysis of human blood

4.1. Introduction

Analysis of the components and cells of the blood has become a standard procedure in medical diagnosis. The number, proportions and shape of the blood cells, the quantities of various ions and proteins, the osmotic pressure, the concentration of various substances and a number of other factors help the physician to draw a correct diagnosis. Analysis of blood is often carried out for research purposes, as a number of diseases and physiological processes (e.g. stress, administration of certain drugs, metabolic effects, etc) alter its characteristic values and composition. The aim of the following exercises is to study some basic phenomena like clotting and bleeding time, to identify the blood group of the students in the lab, to determine the average number of red and white blood cells and to investigate the ratio of basic cell types in blood smears.

Blood is a liquid connective tissue that consists of cells and cell fragments (named formed elements) surrounded by a liquid extracellular matrix (or blood plasma). Blood has three general functions: transport, homeostasis, and protection. It transports respiratory gases (O_2 and CO_2) between lungs and body cells and nutrients, waste products and different regulatory molecules (like hormones) inside our body. Circulation of blood helps maintain the homeostasis of all body fluids, including the regulation of pH, osmolarity or temperature. Blood also protects our body either by clotting and thus, preventing excessive bleeding or can fight diseases or pathogenic intruders.

4.1.1. Components of blood

Blood plasma is a straw-coloured (yellowish) liquid, which makes up about 55% of total blood volume after the sedimentation of formed elements in a blood sample. It contains approx. 91.5% water and 8.5% solutes, most of which are proteins like albumins, globulins or fibrinogen. Antibodies or immunoglobulins belong to the group of globulins, and play important role during certain immune responses. Besides proteins, other solutes like electrolytes, nutrients, waste products, regulatory substances and gases are also present.

Formed elements include three principal components: **red blood cells** (RBCs or erythrocytes; 4.8-5.4 million/µl blood), **white blood cells** (WBCs or leukocytes; 500-1.000/µl blood) and **platelets** (thrombocytes; 150.000-400.000/µl blood). While RBCs and WBCs are whole cells, platelets are only cell fragments.
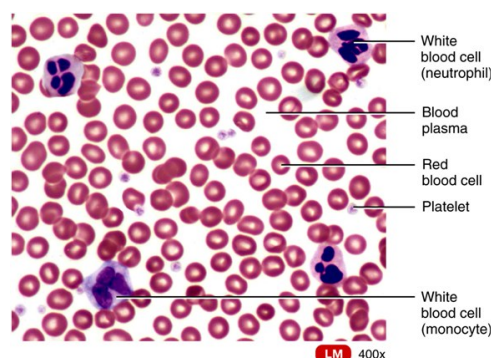
Figure 4.1. *Characteristic formed elements of the blood*

The percentage of total blood volume occupied by formed elements, mostly RBCs is called the **hematocrit**, with a normal range of 38-46% or 40-54% in case of healthy adult females or males, respectively. RBCs are biconcave discs (doughnut shape) with a diameter of 7-8 µm. In their mature form, they lack a nucleus. They contain large amount of hemoglobin molecules, which play an important role in the transport of respiratory gases. RBCs live around 120 days within the circulation. The ruptured cells are removed and destroyed by macrophages in the spleen and liver.

WBCs have nuclei and all cellular organelles and are classified as either granular or aglanular, depending on whether they contain cytoplasmic granules that can be visualised by staining. **Granular leukocytes** include neut-

rophils, eosinophils and basophils, depending on the type of dyes staining their granules. Their nuclei have lobes, connected by thin strands of nuclear material. **Neutrophils** (60-70% of WBCs) are active in phagocytosis, thus, they can ingest bacteria and dispose of dead cellular matter. Besides engulfing bacteria, these cells are able to release several chemicals that help to destroy pathogenic intruders. In case of an inflammation, neutrophils are able to leave the blood stream to fight injury or infection. **Eosinophils** (2-4% of WBCs) are also able to leave the capillaries and enter tissue fluid. They can phagocytize antigen-antibody complexes and are effective against certain parasites. They can also release substances involved in inflammation during allergic reactions. **Basophils** (0.5-1% of WBCs) can also leave capillaries at the sites of inflammations. They release granules that contain heparin, histamin and serotonin, which intensify inflammatory reactions and are involved in hypersensitivity (allergic) conditions.

Agranular leukocytes include lymphocytes and monocytes. **Lymphocytes** (20-25% of WBCs) have a round nucleus which almost completely fills out the cytoplasm. Their average size is 6-14 µm. They can continuously circulate between blood, tissues and lymphatic fluid and under normal conditions, only their 2% is present in the bloodstream at any given time. There are 3 main types of them. **T cells** attack viruses, fungi, some bacteria, transplanted or cancerous cells and are responsible for transfusion reactions and the rejection of transplanted organs. **B cells** produce antibodies and are particularly effective in destroying bacteria and inactivating their toxins. **NK (natural killer) cells** attack a wide variety of infectious microbes and tumorous cells. **Monocytes** (3-8% of WBCs) have nucleus which is kidney- or horseshoe-shaped. They are rare within the circulatory system, as they soon migrate into the tissues, where they enlarge and differentiate into macrophages. Some of these cells are fixed (tissue) macrophages, like in the lungs or in the spleen, while others become wandering macrophages, which gather at sites of tissue infection or inflammation. They clean up cellular debris and microbes by phagocytosis.

Table 4.I. *Significance of high and low white blood cell count.*

WBC type	high count may indicate	low count may indicate
neutrophils	bacterial infection, burns, stress, inflammation	radiation exposure, drug toxicity, vitamin B_{12} deficiency, systemic lupus erythematosus (SLE)
eosinophils	allergic reactions, parasitic infections, autoimmune diseases	drug toxicity, stress
basophils	allergic reactions, leukemias, cancers, hypothyroidism	pregnancy, ovulation, stress, hyperthyroidism
lymphocytes	viral infections, some leukemias	prolonged illness, immunosuppression, treatment with cortisol
monocytes	viral or fungal infections, tuberculosis, some leukemias, other chronic diseases	bone marrow suppression, treatment with cortisol

Platelets are irregularly disc-shaped, 2-4 –µm diameter cellular fragments of megakaryocytes. They have a short life span (4-9 days) in the circulation and then are removed by fixed macrophages in the spleen or liver. They have no nuclei but contain many vesicles which promote blood clotting upon the release of their content. Platelets additionally help stop blood loss by forming a platelet plug in the damaged vessels.

4.1.2. Hemostasis

When blood vessels are damaged or ruptured, a sequence of responses leads to the stop of bleeding (called hemostasis). Blood loss is reduced by three consecutive mechanisms: **vascular spasm**, **platelet plug formation** and **blood clotting**. The first two reactions might be enough for closing small vessels, but excessive blood loss can be prevented only by a complex cascade of enzymatic reactions, leading to the formation of a gel-like clot, containing formed elements of the blood entangled in fibrin threads. Blood clotting can be induced by two pathways, called the extrinsic and intrinsic pathways, which both lead to the conversion of soluble fibrinogen into insoluble fibrin, which then forms the thread of the clot. Once the clot is formed, it plugs the ruptured area of the blood vessel and stops further loss of blood. As the clot retracts and pulls the edges of the damaged vessel closer together, some serum can escape between the fibrin threads but the formed elements are trapped within. The size of the clot is tightly regulated by the fibrinolytic system and some anticoagulants to prevent the formation of intravascular clots or excessive closure of vessels.

When the blood clots easily, it can lead to thrombosis, i.e. the closure of an unbroken blood vessel. If it takes too long for the blood to clot, haemorrhage can occur. Normal clotting is started within 5-6 minutes over inert (e.g. paraffinated) surfaces, but can be much faster on less smooth surfaces. In case of smaller wounds, bleeding stops within 2-3 minutes.

4.1.3. Blood groups and blood types

The surface of the RBCs contains a genetically determined assortment of antigens composed of glycoproteins and glycolipids. Blood is characterised into different **blood group**s, based on the presence or absence of these **antigen**s or **agglutinogen**s. There are more than 24 blood groups and over 100 antigens, but the ABO and the Rh are the most immunogenic ones, so we will discuss only these two.

The **ABO blood group** is characterised by two glycolipid antigens, called A and B – depending on whether the RBCs have none, only one or both antigens, blood groups are distinguished as **type O**, **type A**, **type B**, or **type AB**. Blood plasma contains **antibodies** or **agglutinins** that react with non-self antigens (see Table 4.II). These antibodies are formed soon after birth, in response to bacteria that normally inhabit the gastrointestinal tract, and carry the same antigens. Under normal circumstances, lymphocytes recognize only those antigens that are not present in the child's body. Because the resulting antibodies are large IgM-type molecules that cannot cross the placenta, incompatibility between mother and foetus is not a common problem.

When blood transfusion is needed to alleviate anaemia or to increase blood volume after excessive bleeding, only those donor RBCs can be transplanted into the recipient's body, which are not recognised by the recipient's own antibodies. Should it happen otherwise, a **transfusion reaction** would occur leading to the **agglutination** of the donor RBCs. Agglutination is an antigen-antibody response in which foreign RBCs are cross-linked to each other, leading to the haemolysis of RBCs and kidney malfunction. See Table 4.II. for the interactions between major ABO blood types. Accordingly, type O blood group can provide RBCs to any other ABO blood type as their RBCs do not contain any antigens, while type AB people can receive blood from any other blood group as they do not have any antibodies against RBCs in their blood plasma.

Table 4.II. *Characterization of blood types*

	blood type			
	A	***B***	***AB***	***O***
agglutinogens (antigens) on RBCs	**A**	**B**	**both A and B**	**none**
agglutinin (antibody) in plasma	**anti-B**	**anti-A**	**none**	**anti-A and anti-B**
compatible donor blood types (from which blood type can it receive RBCs)	**A, O**	**B, O**	**A, B, AB, O**	**O**
incompatible donor blood types (when this type of blood is received, hemolysis occurs)	**B, AB**	**A, AB**	**none**	**A, B, AB**

The **Rh blood group** is named after the presence of the **Rh factor** or **D antigen** on the surface of RBCs. People having the D antigen are called **Rh+** (**Rh positive**), while those lacking Rh antigens are regarded as **Rh-** (or **Rh negative**). Normally, blood plasma does not contain anti-D antibody, however, in case an Rh- person receives Rh+ blood, immunisation occurs and anti-D antibodies are produced. If a second transfusion of Rh+ blood takes place, the already formed anti-D antibodies lead to the agglutination of the donor Rh+ RBCs. This is especially critical in case of **Rh incompatibility** between Rh- mothers and their Rh+ fetuses causing the **haemolytic disease of the newborn, HDN**. During the first pregnancy, fetal RBCs are normally isolated from the mother's circulation. During birth, however, maternal and fetal blood can mix which leads to the immunisation of the Rh- mother and thus, to the formation of anti-D antibodies. This normally does not cause any harm to the first Rh+ foetus, but can lead to severe problems in case of the following Rh+ fetuses. As anti-D antibodies can cross the placenta, they lead to the agglutination and haemolysis of the Rh+ fetal RBCs, leading to jaundice, hypoxia and developmental problems.

Fortunately, Rh incompatibility reactions can be prevented nowadays by the **passive immunisation** of Rh- mothers in case of giving birth, miscarriage or abortion. During this process, Rh- mothers are injected with pre-made anti-

D antibodies, which bind to the fetal RBCs. The binding prevents the mother's immune system to recognise the antigen, and to form antibodies against it.
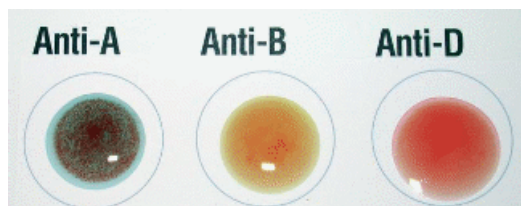
Figure 4.2. *Blood typing for the ABO blood group. Blood sample is agglutinated in the pre-made anti-A sera, while it flows normally in case of anti-B and anti-D sera. Thus, investigated blood sample belongs to type A, Rh-blood group.*

4.1.4. Blood typing

This is a regular clinical test. In this procedure, a drop of blood is mixed with pre-made antisera containing certain antibodies (anti-A, anti-B or anti-D). For the sake of safer interpretation, a mixture of anti-A and anti-B antibodies is also used. In case the RBCs of the tested blood contain the responding antigens (A, B, or D, respectively), pre-made antibodies cross-link them and lead to their agglutination. It is important to emphasize that during blood typing, not the presence of the plasma antibodies but the RBCs' surface antigens is tested (see Figure. 4.2)

4.1.5. Materials needed

Each group needs cotton wool and 70% ethanol to disinfect the fingers; a clean tile, sterile microscopic slides, fingerpricker, ABO and Rh serotypes (anti-A, anti-B, anti-A + anti-B, anti-D antibody solutions), physiological solution (0.9% NaCl), stop watch, blood smears prepared in advance and a microscope. **During the practical, each student should work only with his/her own blood - if help is needed, gloves must be worn! Any equipment getting in contact with human blood has to be first disinfected, and then cleaned afterwards. Used finger-prickers must be collected in the hazardous waste together with any blood-stained paper/cotton.**

4.1.6. Drawing blood from the finger

Disinfect the pad of the middle finger with ethanol. Stop circulation in the middle of the finger with the thumb until the end of the middle finger has deep red colour. Press the fingerpricker on the skin of the middle finger, and press the button releasing the needle. **Throw the used fingerpricker immediately into the hazardous waste!** Wipe off the first drop of blood as it contains a large amount of other tissue fragments, and start drawing blood. If bleeding subsides, massage the finger.

4.2. Quantitative analysis of human blood

In order to count average *red* and *white blood cell number*, cells have to be treated with a fixative, and when required, also stained. For the dilution of blood, automated pipettes (**see Figure. 4.3**) are used.

4.2.1. How to use the pipettes

There are two types of pipettes used for the dilutions: yellow colour indicates a volume between 2-20 µl (P-20), while grey colour marks 100 to 1000 µl volume (P-1000). (These volumes are also indicated at the side of the pipettes). Required volume can be adjusted by turning the volume adjustment knob: in the small window, numbers are displayed accordingly, in the decimal positions. Thus, to pipette 180 µl of blood, set in the window of the 100-1000 µl pipette the 0-1-8-0 numbers. Pipette tips should be used accordingly: they are located in separate boxes for 2-20 µl and 50-1000 µl tips, respectively. To prevent contamination of the pipettes with human blood, tips contain filters and can be used only once! When using the automated pipettes, take care that there are two positions of the plunger when pressed: the desired volume is measured until the first position while full press-down serves for ejecting the total amount of liquid from the tips. Thus, in order to pipette a certain volume, follow these steps:

- Select the appropriate pipette for your desired volume.

- Dial the volume adjustment knob until the desired volume is shown in the small window.

- Firmly place a disposable tip on the shaft of the pipette.

- Press down the plunger to the first stop.

- Hold the pipette vertically while keeping the plunger at the first position and immerse the disposable tip into the liquid up to several mms. Never immerse the entire tip!

- Slowly release the plunger to its original (rest) position. Do not allow it to snap up!

- Withdraw the tip from the sample.

- Place the tip against the wall of the receiving tube- when the receiving tube already contains liquid, immerse the tip into the sample. Push the plunger down to the first stop.

- Depress the plunger to the second stop in order to expel any residual sample in the tip.

- While the plunger is still depressed, remove the pipette from the tube and slowly allow the plunger to return to its original position.

- Discard the disposable tip by pushing the ejector button.

Figure 4.3. Automated pipette with adjustable volume. Yellow colour indicates a volume between 2-20 µl, while grey coloured pipettes can be used between 100 to 1000 µl.

4.2.2. How to make dilutions

First prepare the required fixative into a clean Eppendorf. When it is ready, disinfect and punch the middle finger, wipe off the first blood drop then gently massage the finger to draw a larger drop of blood on the surface of the finger. Immerse a disposable sterile pipette tip attached to the corresponding pipette into the drop of blood – make sure that the pipette tip is not pressed to the skin, otherwise even sucking of the blood is not possible. Take care to avoid air bubbles inside the pipette tips! Once pipetted, blood should be immediately mixed with the already prepared fixatives.

RBCs should be diluted 100 times with a fixative solution, called Hayem solution. This is a clear liquid as due to their high hemoglobin content, RBCs can be visualised in the light microscope without staining. To achieve the appropriate dilution, pipette 990 µl of Hayem solution into an Eppendorf tube with the P-1000 pipette, then mix it with 10 µl blood, measured with the P-20 pipette. Close the tube and mix thoroughly with a vortex and let it stand. In case of **WBCs, cells should be stained and fixed with the Türk solution, diluting the sample 10 times.** Accordingly, pipette 180 µl of Türk solution into an Eppendorf tube with the P-1000 pipette and mix it with 20 µl blood, measured by the P-20 pipette. Close the tube and mix thoroughly with a vortex and let it stand.

4.2.3. How to count the cells

Cells are counted with the aid of a Buerker chamber or hemocytometer, which consists of a thick glass microscope slide with a rectangular indentation that creates a chamber. The chamber is engraved with a laser-etched grid of

perpendicular lines. As the area bounded by the lines is known (0.2x0.2 mm^2 and 0.05x0.05 mm^2 in case of larger and smaller grids, respectively) and the depth of the chamber is also known (0.1 mm), the volume above the grids can be calculated easily (0.004 and 0.00025 µl, respectively). It is therefore possible to count the number of cells or particles in a specific volume of fluid, and thereby calculate the concentration of cells in the fluid overall (Figure 4.4).
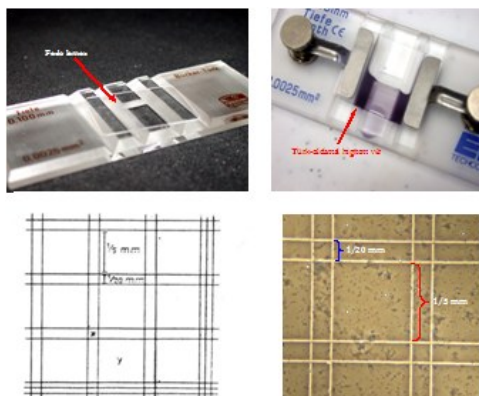
Figure 4.4. *The Buerker chamber. The thickness between the coverslip and the chamber is 0.1 mm; larger squares have 0.2mm, while smaller squares have 0.05 mm side length.*

Once the fixation and dilution is ready, prepare the *Buerker chamber* (Figure 4.4). Put a coverslip over the middle region of the Buerker chamber, where the fine, calibrated network is scratched onto the surface. Vortex the fixated blood samples and pipette a small amount (e.g. 10 µl) from the samples into the slit between the coverslip and the Buerker chamber. During this process, take care that excess solution does not get into the groove between the upper and lower sides. Put the Buerker chamber onto the stage of the microscope and using the 10x objective, count *white blood cells* within 12-12 *large squares* (0.004 µl volume each) from the upper and lower chamber, respectively. Repeat the same procedure for the *red blood cell* counting using the *smaller squares* (0.00025 µl volume each)! During the counting, take care to calculate only those cells which are located entirely within the side lines or are located over two pre-defined side lines (e.g., over the top and left-side lines). Calculate average cell number within the sample volume, and give the final result as **average cell number in 1µl of human blood**.

4.3. Determination of the ABO and Rh blood groups

Drip a drop from each serotype (anti-A, anti-B, anti-A + anti-B, anti-D solutions) and from the physiological solution onto the clean tile and label each of them by the side. Disinfect and stab your finger as it is described above. Unwrap a sterile glass slide, merge one corner into the drop of blood on your finger and mix it with one of the solutions, one after the other. Always use a separate corner for different solutions to prevent mixing different solutions with each other! Once the samples are ready, carefully move the tile to the sides so that blood can mix with the solutions, and wait 1-2 minutes for the appearance of agglutination. Take notes from your findings and decide which blood group your sample belongs to. Explain your conclusion in the lab report!

4.4. Determination of bleeding and clotting time

The bleeding and clotting time indicate the conditions of the blood clotting mechanisms. The latter gives information on the intrinsic clotting mechanisms while the former shows the mutual effects of blood and injured tissue.

Determination of the bleeding time starts with the puncture of the finger pad. Wipe off the blood with a piece of filter paper every 20 seconds, and stop the timer when the wound is dry for the 3rd time. Normal bleeding time is between 2 to 3 minutes.

In order to measure the clotting time, drop the first clear drop of blood onto the inert parafilm (or onto a glass surface covered by paraffin) and start the timer. Draw a sterile needle through the drop in every 20 seconds. Stop

the timer when the first fibrin fibre appears. Normal clotting time is between 5 to 10 minutes on paraffinated surfaces, but can be much faster on less smooth surfaces.

4.5. Qualitative analysis of blood smear

The type, size and characteristics of the formed elements can be observed in thin blood smears with the aid of a microscope. The relative amount of different cell types is normally stable, but can be changed by several diseases. Consequently, the qualitative analyses of different cell types can lead to important conclusions regarding the necessary therapy for patients.

Illuminate the microscope's field of view evenly, then place a numbered blood smear onto the microscopic stage and investigate it with the 40x objective. If needed, the teacher can give you help with the necessary adjustments. Start to look for white blood cells, and moving from one to the other, determine which group they belong to: monocytes, lymphocytes or granulocytes. **Keep on looking for at least 50 white blood cells!** Calculate the relative amount for each cell type and conclude whether the sample shows normal values or might be from an ill person. In case of the latter, try to find an explanation for the observed changes!

Chapter 5. Human electro-cardiography (ECG) and analysis of cardiovascular system

5.1. Introduction

The heart consists of specialized muscle that has the fundamental physiological property of rhythmicity. This feature accounts for the uniformly paced alternating periods of contraction and relaxation. In the human the heartbeat is regulated by the *sinoatrial (SA) node,* located in the wall of the right atrium. After electrical activity spred to the atrial musculature, it is conducted to the ventricular musculature via specialized conducting pathways, including the *atrioventricular (AV) node*, the *bundle of His*, the *right* and *left bundle branches* and *Purkinje fibers* (see *Figure5.1.A*).
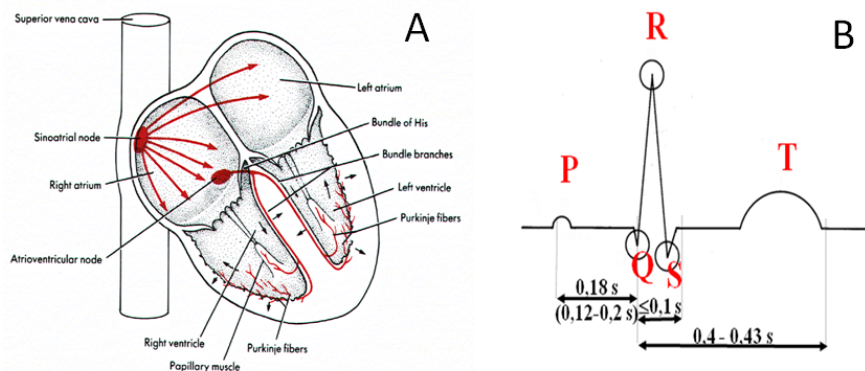
Figure 5.1. *A) The conductive system of the human heart. B) A standard ECG curve with the characteristic waves and peaks.*

When depolarization reaches the contractile cardiac muscle cells, they contract – this mechanical event is called **systole**. After repolarization, cells relax, which is called **diastole**. Thus, the rhythmic change in electrical activity leads to the mechanical pumping action of the heart, providing the driving force for circulating blood.

About 60-70% of the human body is made up by an electrolyte solution, therefore it conducts electricity well and serves as a volume conductor. Therefore, the electric field generated by the electrical activity of the heart can be recorded anywhere on the body surface. The *electrocardiograph* is the instrument that monitors electrical activity of the heart. The record produced by the electrocardiograph is called an *electrocardiogram* abbreviated *ECG* or *EKG*. The ECG is a composite record of the potential changes produced by all cardiac muscle fibers during each heartbeat. Thus, ECG can reflect interruptions of the electrical signal generation or transmission but cannot provide information on pulse volume, the force generated or blood pressure changes, etc.

The component waves of a normal ECG are shown in Figure 5.2.B. The major components are the *P wave,* which indicates the spread of excitation from the sinoatrial node over the atrial musculature (atrial depolarization); the *QRS wave* (or *QRS complex*), which represents the rapid depolarization of the ventricular heart muscle that immediately precedes ventricular systole; and the *T wave,* which represents the repolarization of the ventricular muscle that occurs just before ventricular diastole. The T wave is smaller and wider than the QRS complex because repolarization occurs more slowly than depolarization. During the plateau period of steady depolarization, the ECG tracing is flat. The time spans between waves are called intervals or segments. The *P-Q interval* represents the conduction time required for the action potential to travel through the atria, AV node and other fibers of the conductive system. The *S-T segment* represents the time when the ventricular contractile fibers are depolarized during the plateau phase of the action potential. The *Q-T interval* is the time from the beginning of ventricular depolarization to the end of ventricular repolarization. *Atrial contraction* (or systole) is developed during the peak of the P

wave and lasts until the onset of the Q wave, while *ventricular systole* takes place between the onset of the QRS complex until the peak of the T wave.

In clinical ECG practice, electrodes are positioned on the arms and legs (limb leads) and at six positions on the chest (chest leads). During the practical, the standard Einthoven recording system is to be used that is based on the limb electrodes only. Potential difference is measured between pairs of electrodes that are connected to either the negative or the positive input of a differential amplifier. This setup with two discrete electrodes of opposite polarity is called a *bipolar lead*. The electrode positions for the different leads have been standardized (see *Figure 5.3.A*). Lead I has the negative electrode on the right wrist and the positive electrode on the left wrist; Lead II has a negative electrode on the right wrist and a positive on the left ankle, while Lead III measures electrical activity between the left wrist (negative electrode) and the left ankle (positive electrode). On the right ankle the ground electrode is located. The relationship of the bipolar limb leads is such that the polarity of the ECG curve is the same in all leads, and the sum of the electrical currents recorded in leads I and III equal the electric current recorded in Lead II. This relationship is called *Einthoven's law*. It is easy to deduct that if the currents for any two leads are known, the value for the third lead can be calculated.

The bipolar limb lead axes can be used to construct an equilateral triangle, called *Einthoven's triangle*, at the center of which lies the heart (see *Figure 5.3.B*). Each side of the triangle represents one of the bipolar limb leads, as the physical distance between the electrodes and the heart is approximately the same for all leads. The spread of electric current between the measuring electrodes of a given lead can be represented by a *vector*, which has magnitude, direction and polarity, and is commonly visualized by an arrow. Thus, at any given moment of the cardiac cycle, a vector represents the net electrical activity seen by a lead. The *mean electrical axis* of the heart is the summation of all vectors occurring in a cardiac cycle. Since the QRS complex caused by ventricular depolarization represents the majority of the electrical activity of the heart, adding the QRS wave amplitudes of different leads together approximates the mean electrical axis of the ventricles, that is influenced by the spatial position of the human heart inside the chest, but also by the differences in electrical activity of the left and right halves of the heart.
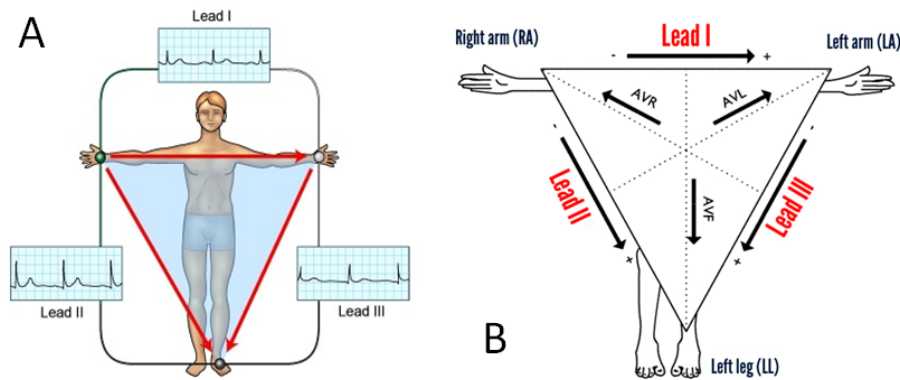
Figure 5.2. *A) The standard bipolar leads and their corresponding ECG curves. Note that the highest amplitude of the QRS complex is measured in Lead II, which is almost parallel to the physical position of the human heart inside the chest. B) A representation of the Einthoven's triangle and a way to calculate it.*

The normal range of the mean electrical axis of the ventricles is approximately -30º (slightly deviating toward the left shoulder – 0º is the horizontal) to +90º (vertical). The axis can shift slightly with a change in body position (like between supine and standing positions) and can depend on individual differences in heart mass, body mass index, and the anatomic distribution of the cardioventricular conductive system. *Left axis deviation* takes place when the mean electrical axis of the QRS complex is shifted between -30º to -90º. This can be a consequence of abnormally delayed depolarization of the left ventricle, caused by muscle hypertrophy (like in case of "sport heart" developed by excessive training or in case of hypertension) or can be caused by damaged ventricular myocardium blocking or slowing depolarization. During pregnancy or in case of excess body fat, the diaphragm is pushed upwards which can also lead to a change in the heart's position. *Right axis deviation* happens when the mean electrical axis of the QRS complex is between +90º to +180º. In some cases, especially in young adults with long and narrow chest or in case of standing, the heart can take a more vertical position. In most cases, however, right axis deviation is related to the hypertrophy or a damaged conductive system of the right ventricle.

The heart and blood vessels form the so-called cardiovascular system, which works as an anatomical and functional unit. In vertebrates, blood flows in a closed circulation, driven by the mechanical pumping action of the heart. Several interconnected feedback systems control blood pressure and blood flow by adjusting heart rate, stroke volume, systemic vascular resistance or blood volume. Some systems allow rapid adjustments to cope with sudden changes (like in case of rapid change between supine to standing position), while others act more slowly to provide long-term regulation of blood pressure or the distribution of blood within the circulation (like in case of extended exercise). The *cardiovascular (CV) center (see Figure 5.3)* is located in the medulla oblongata and regulates heart rate and stroke volume as well as neural, hormonal and local negative feedback systems to control blood pressure and blood flow to specific tissues. Besides central and systemic neural regulation, cardiovascular system is under the influence of *local regulatory actions* (like autoregulation of blood vessels) and *systemic hormones*, including *vasodilators* (kinins, VIP or ANP) or *vasoconstrictors* (vasopressin, norepinephrine and epinephrine, angiotensin II).

Within the CV center, there are neurons which regulate heart rate and contractility of the ventricles as well as blood vessel diameter by increasing or decreasing the frequency of nerve impulses in both the sympathetic and parasympathetic branches of the *autonomous nerve system (ANS)*. In the former case, cardiac accelerator and vasoconstrictor functions are driven by the so-called *pressor neurons* of the CV center, regulating the activity of *sympathetic nerves*. *Depressor neurons* within the CV center, on the other hand, inhibit the action of the pressor neurons and regulate parasympathetic innervation and activity via the *cranial nerve X* (also called *vagus nerve*). The CV center receives inputs from higher brain regions (e.g. from cerebral cortex, limbic system or hypothalamus) and from sensory receptors. These include *proprioceptors*, which monitor the movements of joints and muscles and provide information about physical activity, *baroreceptors*, sensing blood pressure changes, and *chemoreceptors*, monitoring blood acidity (pH) or pO_2 and pCO_2 levels.
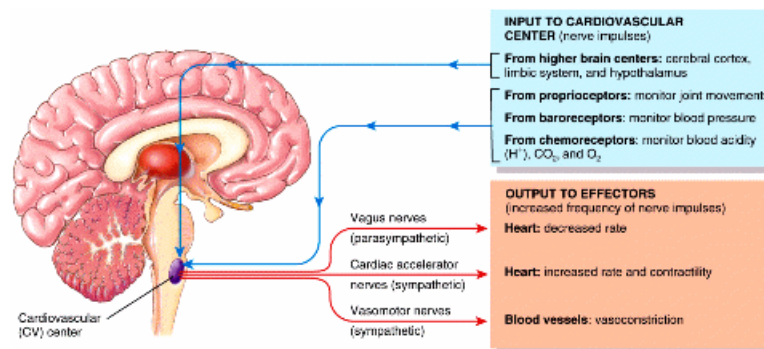
Figure 5.3. *Location and function of the cardiovascular (CV) center in the medulla oblongata. The inputs and outputs of the CV center are highlighted.*

Baroreceptors are pressure-sensitive sensory receptors, located in the aorta (*aortic arch receptors*), in the internal carotid arteries (*carotid sinus receptors*) and some other large arteries in the neck and the chest. They send impulses to the CV center – the higher pressure they measure, the higher frequency of impulses is generated in the sensory axons within the vagus (X) or the glossopharyngeal (IX) nerves, respectively. Afferent impulses activate the depressor neurons of the CV center – thus, increased blood pressure leads to increased rate of baroreceptor afferent impulses, which increases parasympathetic and decreases sympathetic stimulation. As a result, heart rate and the force of contraction are reduced, thus cardiac output is decreased. Furthermore, decreased sympathetic activation leads to vasodilatation, which lowers systemic vascular resistance and consequently, decreases systemic blood pressure. Thus, arterial blood pressure induces a negative feedback regulation on blood pressure via baroreceptor reflexes.

Normal human heart rate is around 70 bpm (beats per minute), which is somewhat lowered during sleep (*bradycardia*) and accelerated (*tachycardia*) by emotional changes, exercise, fever, etc. When healthy young individuals breathe at a relatively slow rate, heart rate can vary according to the phases of respiration: heart rate is somewhat increased during inspiration and decelerates during expiration, especially if the depth of breathing is increased. This is called *normal* (or *respiratory*) *sinus arrhythmia*, which is a normal phenomenon and is caused partly by the increased filling of the heart during inspiration (Hering-Breuer reflex), partly by fluctuations in the parasympathetic output to the heart. During inspiration, the increasing activity of stretch receptors in the lungs inhibits through the vagal nerves the depressor (cardioinhibitory) area in the medulla oblongata. The parasympathetic

vagal tone that keeps the heart rate at a slow pace decreases, and the heart rate rises. This effect ceases after the end of inspiration, thus during expiration, the heart rate is reduced again.

5.2. Measurement of human electrocardiogram (ECG) using the Biopac system

Aim of the practical: During this exercise, students get familiar with bipolar ECG measurements and study ECG curves, identify characteristic ECG waves and determine the mean electrical axis of the ventricles. Basic cardiovascular regulatory reflexes will be also observed and discussed.

Materials needed: Biopac MP30/35/36 unit, two Biopac electrode lead sets (SS2L); disposable electrodes, electrode gel, paper tissue, 70% ethanol.

Experimental setup:

Clean the skin above the inner surface of both wrists and ankles and attach the disposable electrodes as indicated on Figure 5.4. In case of the left wrist and the right ankle, 2-2 electrodes should be attached. Connect the electrodes to the Biopac lead sets, following the colour code:

Electrode lead I – Channel 1: right wrist should have the white (negative), while left wrist should have the red (positive) electrode, right ankle should have the black (ground); Electrode lead III – Channel 2: left wrist should have the white (negative), while left ankle should have the red (positive) electrode and the black (ground) should be attached to the right ankle.
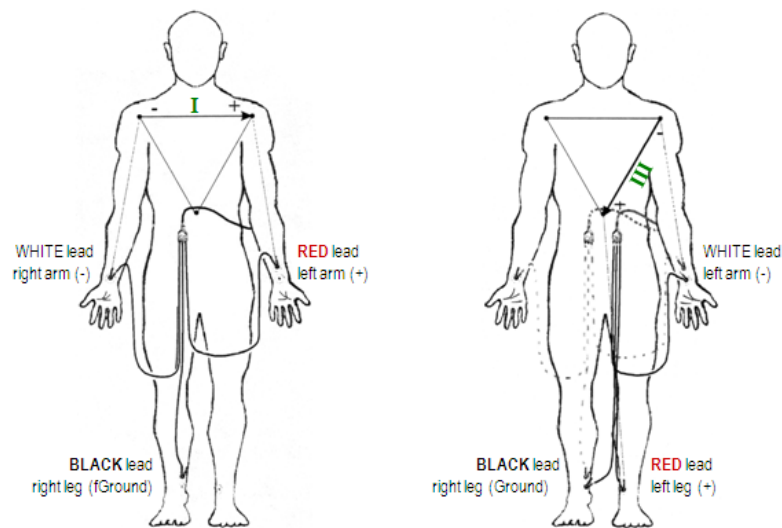
Figure 5.4. Electrode lead connections for Lead I and Lead III.

Switch off the Biopac MP30/35/36 unit and attach the lead sets in the following way (Figure. 5.5):

Lead set I – Channel 1 ------ Lead set III – Channel 2
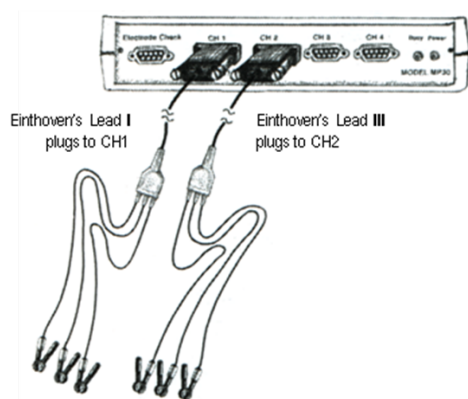
Figure 5.5. Connections of electrode lead sets to the Bipac MP30/35/36 unit.

Position the electrode cables such that they are not pulling on the electrodes. If needed, connect the electrode cable clip to a convenient location on the Subject's clothing. Subject should not be in contact with any nearby metal objects and should remove any wrist or ankle bracelets. Mobile phones should be switched off and should not remain at the Subject! Subject should take a comfortable position and should relax so that muscle EMG signal does not infer with the ECG signal.

Calibration

When the setup is ready, turn on the MP30/35/36 unit and start the Biopac Student Lab program with Lesson 6 (ECG II). Press **Calibrate** button, located in the upper left corner of the Setup window. At the end of the 8-second-long calibration recording, proper ECG curves should be displayed on both channels, with no large baseline drifts. In case it is needed, check that all electrodes make good contact and repeat calibration by clicking the **Redo Calibration** button. In case the calibrations are successful, start the measurement (**Record**). During the recordings, the Subject should not look at his/her ECG curves as this might influence sympathetic activity.

Recording data

During each task, at least 20 to 30 seconds of ECG should be recorded. In case the recording is not satisfactory, you can repeat the given task by clicking the **Redo** button. Once a task is finished, press **Suspend!** The next task can be started upon pressing the **Resume** button – in this case, the new segment is marked by a diamond sign (♦). In case it is needed, a description of the segment can by typed into the textbox above the sign. By pressing **F9**, individual marks can be positioned within the segments, e.g. to mark the onset of inspiration, the change in body position, etc. When all tasks are done, recording can be finished by clicking **Done**.

When any actions are to be carried out during the recordings (like pressing baroreceptors, standing up, etc), make sure to leave sufficient time to record baseline values before executing the given task, and also to continue recording for an appropriate time after the given task is finished to let the values to return to baseline. By pressing F9, individual markers can be saved, as well.

Measurements should be carried out in a relaxed, seated position, unless otherwise indicated.

Experimental tasks

5.2.1 Investigating the effect of body position

Record the Subject's ECG in the **supine** (lying down), **sitting** and **standing** position! Subject should be in the desired position at least 2 minutes before starting the recording. Press Suspend between each tasks and define the segments in the textbox accordingly.

5.2.2. Investigating cardiovascular reflexes

- Find the *carotid artery* on one side of the Subject's neck, *close to the ear*. A helping person should position his/her finger lightly above the carotid artery. Start the recording and after 20-30-second long control segment,

press the artery close to the ears! At the same time, press F9 to mark the onset of pressing. Hold on for at least 30 seconds, then release pressure while still continuing the recording. Don't forget to press F9 again when the pressing is released! After an additional 30 seconds, suspend the recording. Repeat the same procedure, but this time try to find and press the carotid artery *close to the shoulders*! In the first case, pressing should happen above, while in the second case, below the carotid sinus.

• Test the cardiovascular reflex during a *sudden change in body position*, by *standing up fast from a supine position*! Subject should be in a relaxed, supine position, at least 2-3 minutes before starting the recording. After 30 seconds, Subject should stand up as soon as possible. Do not forget to mark standing up by pressing F9! Continue recording for at least a further 30 seconds.

• Demonstrating *normal (respiratory) sinus arrhythmia*. Subject should be in a sitting, relaxed position. Subject should breathe slowly and deeply, inhaling for approximately 3-4 seconds and exhaling for 5-6 seconds (it is a somewhat slower rate than normal, and refers to 5-6 breathing cycles per a minute). The start of the inhalation should be marked by pressing F9 once, while exhalation by pressing F9 twice. Continue recording for at least 2-3 minutes!

• *Valsalva maneuver*. Subject should be in a sitting, relaxed position, breathing normally. After 30 seconds of control recording, Subject should close his/her glottis and exert strong exhalation movements, but without letting the air out. Keep the pressure on for 15-30 seconds and mark the onset and the release of the pressure by pressing F9! After the release of the pressure, continue recording for at least another 30 seconds!

5.2.3. Investigating the effects of exercise

Subject should be in a relaxed, seated position. Record a control segment for 20 to 30 seconds, then suspend recording. Subject should immediately start to make some exercise (like making 20 jumping jacks or squats). Once the task is ready, Subject should sit down and recording should be started immediately (during exercise, muscle movement will generate EMG signals which will masks ECG signals, so it is useless to carry out the recording during the exercise).

5.2.4. Data analysis

Start Lesson 6 and choose the **Review Saved Data** mode. Using the original Lead I and Lead III recordings, the program automatically calculates Lead II, according to Einthoven's law. Therefore 3 channels are displayed on the screen, where Ch1 refers to Lead I, Ch2 to Lead III and Ch40 to the calculated Lead II, respectively.

Set up the display window for optimal viewing of the first data segment (use **Display menu -> Autoscale horizontal, Autoscale waveforms, Zoom** options or the magnifying tool). To measure time periods between individual R waves, set magnification accordingly to be able to measure the R-R time difference reliably. Choose Ch2 and the *delta*T measuring option (*delta*T measures the the difference in time between the beginning and the end points of the selected area)! Select at least 10 consecutive cardiac cycles by the **I-beam tool** and measure individual R-R time differences (Figure. 5.6)! The time difference is displayed in the measurement window above the channels – the value can be copied into the information panel using Ctlr+M option or can be inserted e.g. into an Excel file.

In order to calculate the mean electrical axis of the heart, the amplitudes of the R peaks are also needed. Zoom in and set the channels so that one or two individual cardiac cycle(s) are displayed on the screen. Set the first measurement window to Ch1, and the second window to Ch2 (the others should be set to None).
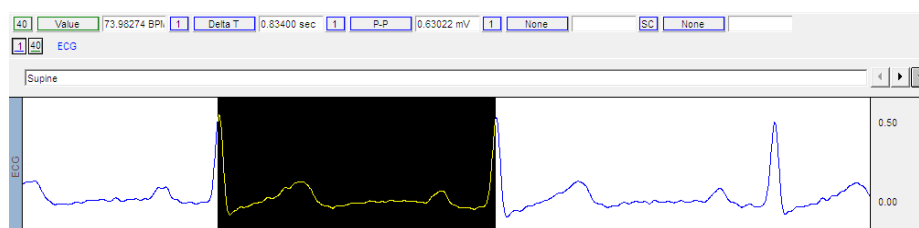
Figure 5.6. *Highlighting R-R time differences with the I-beam tool.*

Choose *delta* for both measurement options (*delta* computes the difference in amplitude between the first point and the last point of the selected area. Take care that the order of selecting the first and last point matters, thus, always mark first the baseline point and the R wave as the second!). Using the I-beam tool, highlight the desired segment as it is shown on Figure. 5.7.
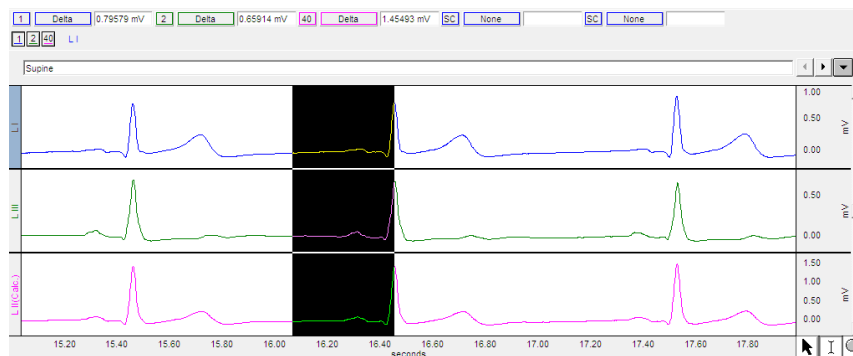
Figure 5.7. Highlighting the amplitudes of R waves.

Using these tools, calculate the following parameters:

• heart rate (beats per minute),

• individual ECG waves and their duration

• atrial conduction time (P-Q interval)

• time needed for atrial and ventricular depolarisation

Do not forget to include the printouts of the recordings in your data report! Make sure that individual segments are correctly marked and the onset of the given tasks is detectable. In case of the cardiovascular reflexes, the best interpretation of data is achieved if the R-R intervals are displayed for each consecutive cardiac cycle in a graph, where cardiac cycles are represented on the x axis, with the corresponding time intervals on the y axis (see Data report for details).

Calculate the Einthoven's triangle in all 3 body positions and determine mean electrical axis of the ventricles! Discuss it whether any changes are expected and whether these changes are reflected accordingly in your recordings.

5.3. Analysis of human blood pressure

In clinical use, the term blood pressure usually refers to the pressure in arteries generated by the left ventricule during systole (systolic value) and the pressure remaining in the arteries when the ventricule is at the end of the diastole (diastolic value). Blood pressure is usually measured in the brachial artery in the left arm (see Figure..5.8). The device used to measure blood pressure is a **sphygmomanometer** [*sphygmo*=pulse; *manometer*=instrument used to measure pressure]. It consists of an inflatable rubber cuff and a meter that registers the pressure in the cuff. During the measurement, the arm should be layed on a table so that it is approximately at the same level as the heart is and the cuff is positioned around the bare arm. Inflating the cuff will compress the brachial artery, leading to a transient stop of blood flow through the compressed blood vessels. As the pressure is gradually decreased within the cuff by slow deflation, the artery will open and a spurt of blood passes through at the peak of the systole, generating a sound. If a stethoscope is placed below the cuff and over the brachial artery, the opening of the artery and the onset of blood flow within the brachial artery as well as the end of the turbulent blood flow can be heard. In case automated blood pressure monitors are used, then a small sensor located within the cuff can sense the small vibrations emanating form the turbulent blood flow inside the brachial artery.The outside pressure within the cuff which enables the first spurt of blood corresponds to the **systolic blood pressure**, the force of blood pressure generated on arterial walls just after ventricular contraction. As the cuff is further deflated, the sound of the turbulent blood flow inside the still partially compressed artery becomes weaker and weaker until blood can flow smoothly through the totally opened brachial artery. This equals to the **diastolic blood pressure**, which represent the force exerted by the blood remaining in the arteries during ventricular relaxation. Instruments also measure **pulse rate**,

which is the number of heart cycles within a minute ans are given as beat per minute (BPM) value. Pulse rate is indicated during the measurement by the "beep" sounds.

In order to carry out measurements with an automated sphygmometer in a reproducible way, the followings should be observed:

1. Subject should sit (or stay in the desired postion) at least for 2 minutes before the measurement.

2. The inflatable cuff should be on the bare upper arm in a way that the tube runs above the brachial artery, along the crook of the arm. Upper arm should not be confined by upturned sleeves.

3. Subject should not move or talk during the measurements.

4. Noise from the environment should be minimalised.

5. Mesurements should be repeated 3 times, in a consequtive manner.
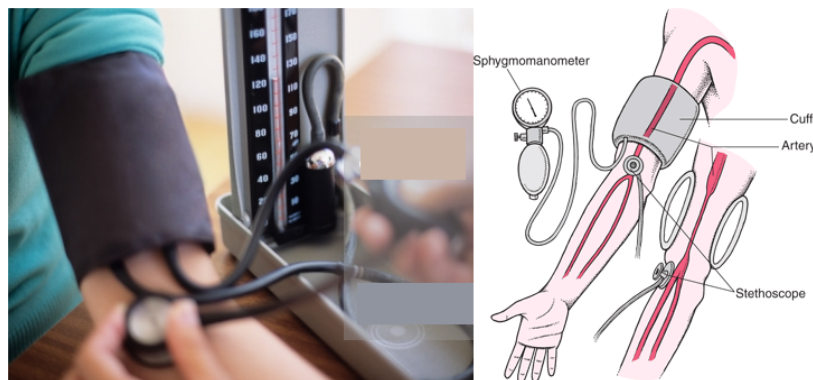
Figure 5.8. *Schematic drawing of a traditional sphygmomanometer and blood pressure measurement.*

Tasks:

1. Record and compare systolic and dyastolic blood pressure in the left arm together with heart frequency (pulse rate) in the following body positions:

    a. supine (lying down)

    b. sitting

    c. standing

2. Record and analyse blood pressure and pulse rate values in a sitting position, directly following excersize (e.g. after 20 squats / push ups).

3. Compute and compare mean Arterial Pressure (MAP) under different experimental conditions of rest and exercise! MAP can be calculated as the value of (systolic blood pressure + 2x diastolic blood pressure)/3.

Measured and calculated values should be recorded in the "Blood pressure" data report, along with explanations of the observed differences.

Chapter 6. Investigating the human respiration

6.1. Introduction

The respiratory system provides three primary functions: it is responsible for the sufficient oxygen supply needed by all cells in the body, help to maintain the pH of the blood plasma providing an outlet for CO_2 and takes important role in the regulation of body temperature, too. The pulmonary system achieves these multiple roles in conjunction with the circulatory system.

Breathing is controlled by neural and chemical regulatory systems. The neurons located in the pons and medulla sends efferent outputs to the respiratory motor neurons located in the spinal cord. The effects of changes in blood gases (pO_2 and pCO_2 levels) on ventilation are mediated via respiratory chemoreceptors, located either in the medulla (centrally) or in the carotid and aortic bodies (peripheral receptors). The purpose of the practical is to observe and investigate some aspects of human respiration and to test some factors influencing respiratory activity.

6.1.1. The respiratory system

The respiratory system consists of a number of anatomical passageways through which the air we breathe travels to the delicately structured lungs. During *inspiration,* meaning the movement of air from the atmosphere into the lungs, air is moved through the respiratory tract (nose, pharynx, larynx) and the conducting airways (trachea and successively smaller divisions of the bronchial tree) until it reaches the numerous tiny air sacs, the pulmonary alveoli. Conditions at the alveoli are ideal for the physiological exchanges of gases between the inspired air and the blood. During *expiration*, the movement of air from the lungs into the atmosphere, the path is reversed. Air flows through these passageways during respiratory cycle due to a pressure gradient generated by the function of respiratory muscles. During *inspiration* the contraction of the diaphragm and the external intercostal muscles increases the volume of the thoracic cavity. The lungs are connected to the chest wall through the double layer of the pleurae, with the visceral pleura being attached to the surface of the lung, and the parietal one to the chest wall. Between the two pleurae, there is a thin layer of fluid. The pressure in this *intrapleural space* is subatmospheric; therefore the lungs are attached to the chest wall and follow the movement of the chest passively. The increased volume of the chest creates less pressure than the atmosphere in the *intrapulmonary space*, so air rushes into the lungs. The lungs are stretched when they expand at birth, and their have a tendency to collapse from the chest wall due to the elastic elements of the lung tissue and the surface tension within the alveoli. The collapse tendency is just balanced by the tendency of the chest wall to recoil in the opposite direction and by the surfactant molecules decreasing surface tension in the alveoli. Therefore, if the chest wall is opened, the lungs collapse and do not follow anymore the movement of the chest as observable in pneumothorax.

Opposite to the active process of the inspiration, *expiration* - during quiet breathing - is mostly passive. After relaxation of inspiratory muscles, the volume of the thoracic cavity decreases, pressuring the gas out of the lungs. This process is driven by the collapsing tendency of the lungs. During exercise or forced exhalation (e.g. coughing), expiration becomes an active process, due to the action of expiratory muscles. Internal intercostals pull down the chest and compress the lungs, while abdominal muscles push the diaphragm upwards.

6.1.2. Measuring the respiratory function

An important quantitation of human respiratory function is the measurement of lung volumes and capacities together with the rate of airflow by the **spirometer** (spiro = breath, meter = to measure). Lung capacities refer to subdivisions that contain some volumes. Diagnostic spirometry is used to assess a patient's lung function for comparison with reference values calculated from the normal population, or with previous measures from the same patient. The *static lung volumes* are independent from time, reflecting the amount of inspired and expired air and providing information about the strength of the respiratory muscles and other aspects of pulmonary functions. On the other hand, *dynamic measurement*s of pulmonary function collect data from the flow rate of expired and inspired air and reflect the state of the airways.

The amount of air that moves into the lungs with each inspiration (or the amount that moves out with each expiration) during breathing is called the **tidal volume (TV, Figure 6.1).** Typical values for TV are *500–750 ml* at rest, but during exercise the TV can be more than 3 l. The air inspired with a maximal inspiratory effort at the end of a tidal inspiration at rest is the **inspiratory reserve volume (IRV)**, which is typically *3100 and 1900 ml* for males and for females, respectively. The volume exhaled by an active expiratory effort at the end of a passive tidal expiration is **expiratory reserve volume (ERV),** which is normally *1200 and 800 ml* in males and females. Some air left in the lungs after an expiration with maximal effort. Average size of this **residual** volume **(RV)** in adults is 1100-1200 ml. Residual volume reflects the fact that after birth, when the lungs have been filled first with air, they are never completely emptied. No expiration ever completely empties the alveoli.

The pulmonary capacities are the sum of two or more primary lung volumes. When all four of the above components (TV+ERV+IRV+RV) are taken together, they make up the **total lung capacity** (TLC, ~5 L). It is important to know, that TLC cannot be measured by spirometry because the residual volume is undetectable with this measurement type.

The total lung capacity can be broken down into alternative capacities that help to define functioning of the lungs. The **vital capacity (VC)** refers to the maximum amount of air expired from the fully inflated lung, or maximum inspiratory level (TV + IRV + ERV). VC is depending on the height (H), age (A) and sex of the investigated person and the following equation can be used for its prediction in males and females:

female: VC=0.052H-0.022A-3.60,   *male:* VC=0.041H-0.018A-2.69

VC increase in adolescence and decrease after reaching the final body size. Of course, vital capacity is also reflecting other characteristics of the individual (e.g. race), therefore 80% of the calculated values are considered as normal.

One of the other calculated capacities is the **inspiratory capacity** (**IC**, ~2.5 L), which is the maximum amount of air that can be inspired from the resting end-expiratory level (IRV + TV). The **functional residual capacity** (**FRC**; ~2.5 L) represents the volume of the air remaining in the lungs after expiration of a normal, resting breath (RV + ERV, Figure. 6.1).

Static pulmonary volumes and capacities are generally measured to assess the health of the respiratory system because pulmonary diseases can fundamentally affect these values. In *restrictive pulmonary diseases* a significant reduction can be observed in the person's ability to inflate and deflate the lungs, therefore some lung volumes and capacities (e.g. VC) are below normal. A variety of disease processes cause restrictive pulmonary disturbances: i) reduction in the respiratory surface, ii) changes or inhibitions of the respiratory movements or iii) reduction in the elastic properties of the lung tissue. The reduction in the respiratory surface and the rigidity of the lung tissue are generally caused by various interstitial lung diseases (e.g, alveolitis, pulmonary fibrosis) by inducing inflammatory, fibrotic or neoplastic processes. The final result is the observable degradation of lung tissue, disappearance of the elastic fibers and accumulation of collagen fibers (scarring). Inhibition of the respiratory muscles due to chest deformity or myo-neuropathic disorders (e.g., Guillain-Barre syndrome, myasthenia gravis, dermatomyositis) also results in changes in the static pulmonary volumes.
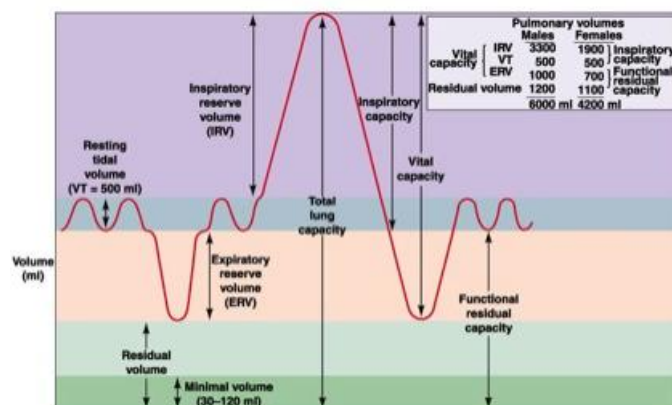
Figure 6.1. *The static pulmonary volumes*

Dynamic measurements of lung volumes and capacities also provide medically useful information to help determine lung dysfunction by measuring the speed of the volume changes in forced breathing. **Forced vital capacity (FVC)**, the largest amount of air that can be expired after a maximal inspiratory effort, is a frequently measured index. It gives useful information about the strength of the respiratory muscles and other aspects of pulmonary function. The major fraction of VC is already exhaled in the first second of forced exhalation maneuver. This volume is termed as FEV_1 (**forced expiratory volume in 1 sec**). Since FEV depends on the personal physical abilities, the FEV_1 to FVC ratio (FEV_1/FVC) is used in practice to compare FEV values of different persons and to recognize the classes of airway disease.

Examination of the FEV values is useful in the detection of *obstructive pulmonary diseases*, such as chronic bronchitis, asthma, or emphysema, which are characterized by an increased airway resistance and FEV value reduction. For example, in *asthma*, the inflammation of the lining of the airways (airway mucosa) and the heavy mucus secretion reduce the airway diameter and therefore increase the airway resistance. This results in episodic or chronic wheezing, cough, and a feeling of tightness in the chest as a result of bronchoconstriction. In *emphysema* the destruction of lung tissue around alveoli makes these air sacs unable to hold their functional shape upon exhalation. The general phenomenon of obstructive diseases is that they are difficult to classify and that environmental hazards (smoking, air pollution, occupational hazards) are important in their generation.

The **maximal voluntary ventilation (MMV)** is the largest volume of gas that can be moved into and out of the lungs in 1 min by voluntary effort. Typically this is measured over a 15 s period and prorated to a minute; normal values range from 140 to 180 l/min for healthy adult males.

Diseases of the respiratory muscles, but an increased airway resistance also might affect the MMV. So changes in MVV in a patient can be indicative for lung dysfunction.

6.1.3. Control of respiration

Various factors are known to regulate respiration. For example, the temperature of blood flowing through the respiratory center may influence breathing. When body temperature is above the normal value, as during fever or severe muscular exercise, respiratory rate (number of breaths per minute) increases. Subnormal temperature, as during hypothermia, results in a decreased respiratory rate. Other respiration regulating factors include pain and emotional state.

Among others, there are two main mechanisms regulating respiration: the neuronal and the chemical control of the breathing. In the medulla, a group of pacemaker cells are responsible for the generation of rhythmic discharges of motor neurons innervating respiratory muscles. These rhythmic discharges produce spontaneous respiration that is influenced by vegetative afferents from the chemo- and mechanoreceptors.

The volume of the inspired air controls the depth and the amplitude of the respiration. This is monitored by mechanoreceptors located in the lung tissue. One type is named as „**stretch receptor**". The activity of these receptors gradually increases when the stretch of the lung increases. This information is transmitted to the respiratory centers in the medulla oblongata by the vagus nerves (n. X.).

Blood gas concentrations (partial pressures) are monitored by chemoreceptors located in the carotic body and in the aortic body (Figure. 6.2). The main function of these receptors is to monitor the oxygen level of the blood. However, in isolated conditions, they can produce a stronger response to changes in the partial pressure of CO_2. The level of CO_2 is also detected by central chemoreceptors located in the ventral part of the brain stem, near to the respiratory centers. Thus, respiration is controlled mainly by changes of the CO_2 level. O_2 is important only when the level of this gas drops below a certain threshold value. Receptors in the carotic body are more sensible to the O_2 level than those in the aortic body. This is because the appropriate O_2 level in the brain is very important.
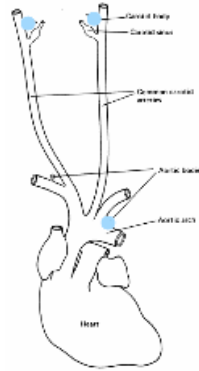
Figure 6.2. Mechano- and chemoreceptors in the aorta and in the carotid arteries.

6.2. Investigation of human pulmonary function

Aim of the practical: Measure and analyze the volumes and capacities of the human lung

Used materials: Computer controlled spirometer and data acquisition software (Piston XP), mouthpiece with bacteria filter, nose clip.

Performing the measurement: After startingthe Piston XP medical diagnostic software suite (Figure. 6.3), press the New Patient button 👥 on the Main Window to enter a new patient into the database. Complete the fields and make sure that the new patient has an individual, dedicated identifier. *Keep in mind, that giving the correct personal data is fundamental to the precise measurement, as the calculation of several variables related to respiratory functions are based on the subject's physical parameters!* To store the patient's data, press the **[Save]** button (💾). You will receive feedback about the success of saving the data.

PDD-301/sh (PinkFlow, Piston Ltd.) computerized flow meter (spirometer) is used to mesure the pulmonary capacities and functions. PinkFlow is a symmetric Pitot tube flow meter, which detects pressure differences that are proportional to the flow speed. A pressure sensor converts the pressure difference to electric signal. The device must be **calibrated** prior to the measurements to ensure the maximum accuracy and it also provides an efficient way to check the proper operation of the device. It is possible to perform measurements without calibration, but at least 5% additional error must be taken into account.
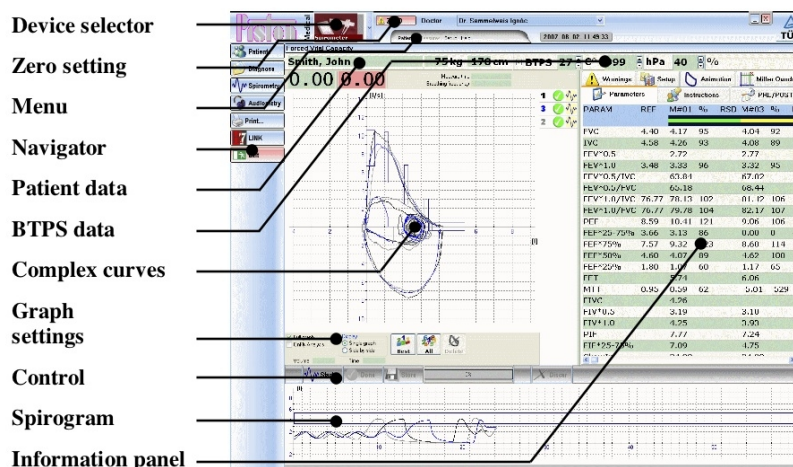
Figure 6.3. The general design of the measurement screen. Some important elements: Zero knob: Runs manual Zero setting of the selected device. Without manual Zero setting the system automatically sets zero before all

measurements Navigator: This field contains control buttons most often used during the daily routine. Control: This field contains control buttons most often used during measurement

For calibration, connect the bigger diameter side of the PinkFlow (patient side) Spirometer directly to the calibrating pump (Figure. 6.4). The calibration should be performed in two steps. At first the peak flow should be at about 1.0 L/s then at about 5.0 L/s. Pull back the plunger of the pump to the end. then press the **Start** button in the [Spirometry/Calibration menu] to start the calibration. Push the plunger into the calibrating pump with uniform speed all the way in, then pull it out all the way. A horizontal line on the loop curve indicates optimal flow limits. During calibration make sure that the calibration curve peaks are within these lines. The first part of calibration (5 cycles) should be done with the peak flow at about 1,0 l/s (red curves). The second part of calibration (5 cycles) should be done with the peak flow at about 5,0 l/s (green curves). After the calibration process the system automatically calculates calibration factors for the different flow values. Results of the calibration can be saved by pressing the **[Store]** button.

Figure 6.4. *Proper connection of the flow meter to the calibrating pump.*

The calibration should be performed at least twice a day. Calibration is also recommended after flow sensor disinfections or replacement. If conditions of work environment (temperature, air pressure, humidity) change significantly, re-calibration is recommended.

For each subject, use a clean PinkFlow flow meter or a new disposable bacterial and viral filter to avoid cross contamination. In order to avoid any nasal breathing during the test apply a nose clip on the investigated subject.

For exact volume measurements, baseline of the flow meter channel must be set to zero immediately before the measurement. The program automatically performs the zero setting process before each measurement. It is important, that during zero setting no air should flow through the flow meter, so the subject cannot take the connected mouthpiece into his/her mouth. Manual zero setting for the currently selected device is also possible with the **[Zero]** button next to the **[Device selection]** list in the program header.

After the calibration, the necessary measurement type: forced vital capacity (FVC), static vital capacity (IVC) or maximal voluntary ventilation (MVV) can be selected with the appropriate button on the Navigation bar.

The collected data and the calculated results can be printed by pressing the **[Print]** button on the Navigation bar or can be exported as a PDF file from the Print menu.

Experimental objectives

6.2.1. Task 1 – Measurement of Static (Inspirational) Vital Capacity

The goal of the measurement is to get the volume of the maximal inspirational capacity. The subject should be instructed to perform the following maneuvers: i) Put on the nasal clip, to prevent nasal breathing. ii) Breathe at least three quiet cycles, then perform a deep expiration, as complete as possible followed by a deep inspiration as deep as possible (Figure. 6.5). The subject has 60 second to perform the IVC maneuver. The **[Done]** button is useful to stop recording, while the **[Discard]** button deletes the collected data. If it is necessary, the measurement

can be repeated by several times, and the most successful of them can be selected for saving by pressing the [Store] button.

The flow meter measures the following parameters in this operating mode:

TV: Resting Tidal Volume, *ERV:* Expiratory Reserve Volume, *IRV*: Inspiratory Reserve Volume, IVC: Inspiratory Vital Capacity, SVC: Static Vital Capacity
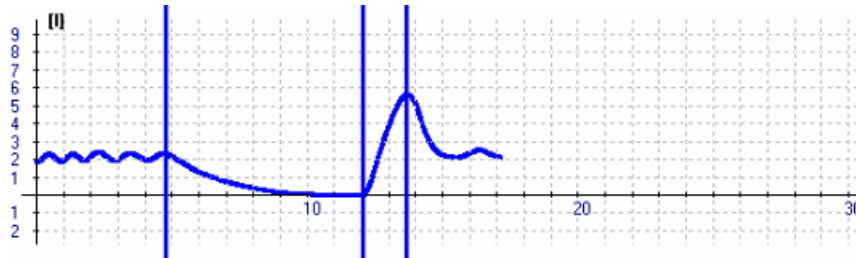
Figure 6.5. *Correct ICV maneuver Phases: Quiet breathing, complete deep expiration, complete deep inspiration, return to normal breathing.*

6.2.2. Task 2 - Forced inspiration/expiration - dynamic recordings

The goal of the measurement is to get the volume and speed of forced expiration and inspiration. The subject should be instructed to perform the following maneuvers: i) Put on the nasal clip, to prevent nasal breathing. ii) Breathe at least three quiet cycles and take an *"as deep as possible"* inspiration followed by an *"as fast and deep as possible"* expiration then an *"as fast and deep as possible"* inspiration (Figure. 6.6). The subject has 60 second to perform the FVC maneuver. In the Animation tab a blowing away dandelions helps with motivating patients (mostly children) to reach their maximum effort during FVC maneuver. The **[Done]** button is useful to stop recording, while the **[Discard]** button deletes the collected data. If it is necessary, the measurement can be repeated by several times, and the most successful of them can be selected to save by pressing the [Store] button.
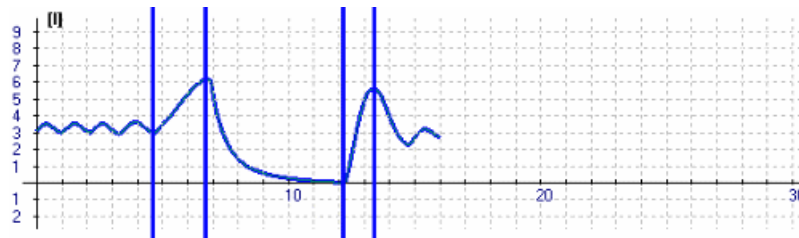
Figure 6.6. *Correct FVC maneuver Phases: quiet breathing, deep inspiration, forced exhalation, forced inhalation, return to normal breathing.*

The flow meter measures the following parameters in this operating mode:

FVC: Forced Vital Capacity.

FEV*0.5: Forced Expiratory Volume 0,5 sec - the fraction of the vital capacity expired during the first 0,5 second of a forced expiration.

FEV*1.0: Forced Expiratory Volume 1 sec - the fraction of the vital capacity expired during the first 1 second of a forced expiration.

FEV*0.5/FVC: The ratio of FEV*0.5 and the forced vital capacity

FEV*1.0/FVC: The ratio of FEV*1.0 and the forced vital capacity

6.2.3. Task 3 - Measurement of maximal voluntary ventilation (MMV)

The goal of the measurement is to get the amount of air the subject can move in a given time. The subject should be instructed to perform the following maneuvers: i) Put on the nasal clip, to prevent nasal breathing. ii) Start breathing moving as much air as possible. The subject can choose the frequency of breathing at his/her convenience. To prevent hypocapnia, it is not recommended to continue this measurement for more than 15 seconds (Figure. 6.7). The subject has 60 second to perform the MMV maneuver. The **[Done]** button is useful to stop recording, while the **[Discard]** button deletes the collected data. If it is necessary, the measurement can be repeated by several times, and the most successful of them can be selected to save by pressing the [Store] button.
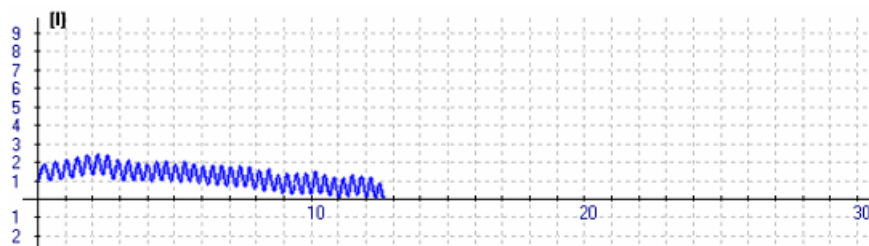
Figure 6.7. *Correct MVV measurement. The subject breathes quickly, evenly during the measurement.*

The flow meter measures the following parameters in this operating mode:

MVV (Maximal Voluntary Ventilation): The maximum respiratory volume measured during voluntary respiration, calculated for one minute

MVV*f (Maximal Voluntary Ventilation Frequency): The maximum respiratory frequency measured during voluntary respiration, projected for one minute

In the comparative study, the measurements should be carried out on more subjects. If it is possible, data from **i) short and tall people from same sex**, data from **ii) males and females with same body mass** and data from **iii) smoker and non-smoker (or with and without allergy or asthma) subjects with the same body mass from the same sex** should be collected.

Analysis and data report

- Compare the results of a tall and a short subject from the same sex!

- Compare the results from a male and a female subject with approximately the same body height and mass!

- Compare the values from a smoking and a non-smoking subject's recording!

- Explain your results!

6.3. Analyzing CO2 content of exhaled air with the Müller's method

Aim of the practical: demonstration of the CO_2 content of the exhaled air.

Materials and tools needed for the practical: 2 gas-washing bottles, mouthpiece, rubber tubes, $Ca(OH)_2$ solution, phenolphtalein, nose clip.

Carrying out the experiment: the simplest *Müller's device* can be constructed from 2 gas-washing bottles filled with $Ca(OH)_2$ solution (see Figure. 6.8). Colour the $Ca(OH)_2$ solution with a drop of alcoholic phenolphtalein! After putting on the nose clip, the subject should breathe in and out continually through the valves of the Müller's

device. The small amount of CO_2 present in the ambient air will be bound to $Ca(OH)_2$ in the right-side bottle (Figure. 6.8), while the CO_2 content of the exhaled air will be detected in the bottle on the left.
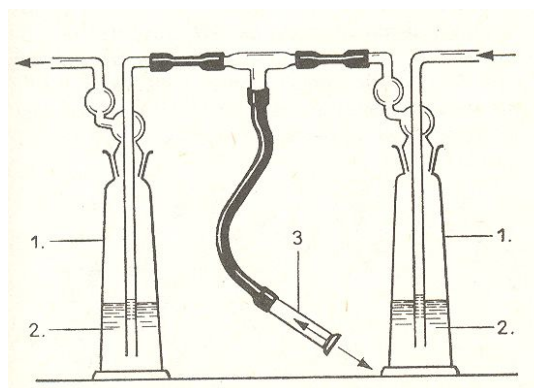
Figure 6.8. *Schematic drawing of the Müller's device. 1: gas-washing bottle, 2: $Ca(OH)_2$ solution coloured with phenolphtalein. 3: mouthpiece.*

Task: Observe the change in the left bottle's colour after a few expirations! Note the indicator's colour change in function of the number of expirations.

Written report: Explain your observations; write down the equations of the chemical reactions underlying the observed phenomena!

Chapter 7. Investigation of human skeletal muscle functions

7.1. Introduction

Skeletal muscle composes the majority of the human muscle tissues. Its name derives from the fact that through tendons it is generally attached to the skeleton (except, for example on the head). Its function is to enable voluntary movements and to maintain the posture, by applying force to bones connected by joints; via contraction. Human skeletal muscles consist of ten to few thousand muscle fibers fused from individual cells during development and fasciculated together by connective tissue. The contractile elements of muscle fibers are the myofilaments formed by chains of sarcomers, and arranged in bundles within the sarcoplasm of the fibers. Muscle fibers show well-developed cross-striation at the microscopic level due to the regular alignment of sarcomers that show A and I stripes because of the arrangement of actin and myosin filaments. The skeletal (or striated) muscles do not have spontaneous activity. Motor neurons in the spinal cord and brain stem control the muscle function, which is generally under voluntary control.

7.1.1. Control of muscle activity and tension

Each muscle fiber is innervated by only one motor neuron, but a single motor neuron can establish several neuromuscular connections. If a motor neuron in the spinal cord is activated, contraction occurs in all innervated skeletal muscle fibers. The combination of a single motor neuron and all of the muscle fibers that it controls is called a **motor unit** (Figure. 7.1). The number of muscle fibers in a motor unit varies. In muscles concerned with fine, precise movement (e.g. muscles that control movements of the eyes or fingers), each motor unit contains 3-6 muscle fibers. On the other hand, motor units in the legs include up to 600 fibers. Although the size of the motor unit usually depends on the function of the muscle, a given muscle can also contain motor units with different sizes and the group of muscle fibers that contribute to a motor unit can be intermixed within a muscle.
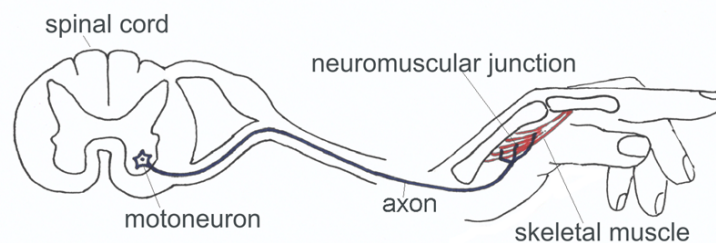
Figure 7.1. A muscle performing the index finger flexion and its motor neuron

Physiologically, the degree of skeletal muscle contraction is controlled i) by the firing frequency of motor neurons in each motor unit, and ii) by the number of activated motor units in the muscle.

A single action potential in the muscle fiber causes a **twitch**, a brief contraction followed by relaxation (Figure. 7.2.A). If an action potential train reaches the fiber due to the repeated firing of the motor neuron, and the action potentials arrive before the fiber can completely relax after the previous contraction, additional activation of the contractile elements occurs and the response (force of contraction) is added to the contraction already present (**summation of contraction**). As a result of rapidly repeated stimulation, the individual muscle twitches fuse into one continuous contraction, which is called **tetanus or tetanic contraction**. It is called **complete tetanus** when no relaxation occurs between stimuli and **incomplete tetanus** when periods of incomplete relaxation take place between the summated stimuli. The force of the contraction in complete tetanus is about four times larger then the one that developes during the isolated twitches (Figure. 7.2.B).

The rate of a muscle contraction also depends on the number of the activated motor units. The process by which muscle strength is enhanced by sequential activation of motor units, is called **recruitment**.
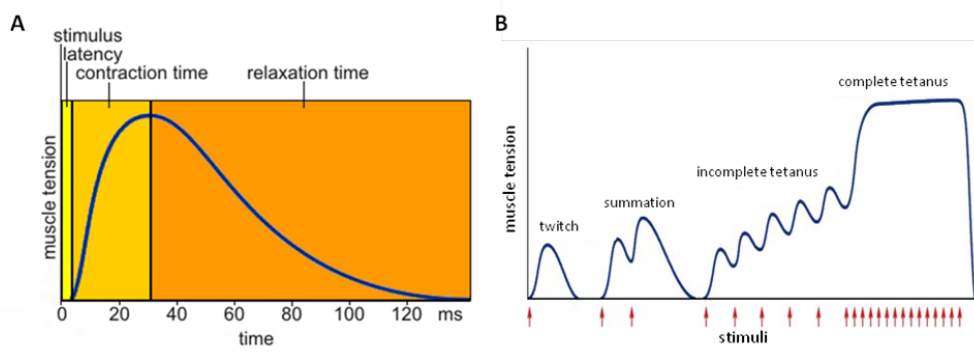
Figure 7.2. A: Single twitch. B: Summation of contraction and tetanus. The red arrows indicate the stimuli. As shown, the tetanus not only causes the fusion of muscle contractions, but also increases the muscle tension.

Muscle fibers can be classified into groups based on their anatomical and physiological characteristics. The slow oxidative fibers (type I) are thin, contain many mitochondria and myoglobin, but less muscle protein and glycogen. The fast glycolytic (type IIb) fibers are thick, fast and have few mitochondria and myoglobin but more muscle protein and glycogen. The third (type IIa) fiber type shows an intermediate phenotype between the two above. It is important to know, that each spinal motor neuron innervates only one type of muscle fiber, thus all muscle fibers in a motor unit are of the same type.

The recruitment of motor units during muscle contraction is not random, but follows a general scheme, the **size principle.** In general, a specific muscle action is developed first by the recruitment of small motor units containing oxidative fibers, because the threshold and the size of corresponding motor neuron are smaller. This is advantageous for two reasons: firstly, the fine movements can be controlled more precisely, secondly, due to oxidative ATP production, these fibers less easily fatigue. Finally, for the most demanding tasks, the fast glycolytic fibers are recruited.

Resting skeletal muscles in humans exhibit a constant state of slight tension called **tone** that serves to maintain the muscle in a state of readiness. The tone is caused by alternate periodic activation of a small number of motor units within the muscle by motor centers in the brain or spinal cord.

7.1.2. Fatigue

If a muscle repeatedly performs maximal acute or chronic submaximal work, the force of the contractions gradually decreases. In most cases, accumulating anaerobic metabolites, oxygen deficiency in the tissue or the failure of the oxidative phosphorylation is responsible for fatigue of the muscle fibers. Since muscle contraction compresses blood vessels supplying the muscle fibers, tissue oxygenation dramatically decreases and some fibers begin anaerobic metabolism. However, red muscles have large oxygen storage ability (due to their myoglobin content) and are capable to maintain aerobic metabolism, even in this case. Fatigue is characterized by the **fatigue time**: the time, in which the force generated by the maximum tension of the muscle is reduced by half. In some neuromuscular disorders (e.g., myasthenia gravis), fatigue time is significantly shorter than normal.

7.2. Monitoring of muscle activity by electromyography (EMG)

Electromyography (EMG) is a technique to record the sum of electrical signals generated by activated muscle fibers during muscle contraction. Most of this electrical activity is the result of by action potentials spreading along sarcolemmal membranes. Action potentials are produced by synaptic activity of motor neurons, as explained above. This electric activity can be measured in unanaesthetized humans by using small metal macroelectrodes on the skin overlying the muscle or by using needle or fine wire electrodes inserted into the muscle among (but not within) muscle fibers. Needle or fine wire electrodes can record the activity of a single or a few (5-10) muscle fibers. Surface electrodes are providing a more integrated view representing the activity of 100-1000 muscle fibers.

The record obtained with such electrodes is the **electromyogram.** The measured EMG depicts the potential difference between the two electrodes, which is altered by the activation of muscles in between the electrodes.

The amplitude of the integrated EMG signal obtained by the rectification of recorded EMG signal is ultimately related to the number of activated motor units providing a useful parameter in practice. If the force of a single muscle is increasing, more motor units are activated (recruitment), so the amplitude of the integrated EMG signal is also increasing. The increase of the EMG amplitude also depends on the strength of the muscle. In a trained muscle, individual muscle fibers contain more myofilaments, thus they are stronger than those in untrained muscles. Therefore, in a trained person, less motor units have to be activated to develop a given force than in untrained person, thus the measured EMG signal will be smaller.

In the clinical practice, EMG is often used to decide whether weakness seen in a patient's muscle is due to a disorder affecting the muscles (myopathy) or the peripheral nerves (neuropathy). The amplitude of the integrated EMG signal and the shape of recorded individual motor unit potentials help to choose between these possibilities. The amplitude of the EMG signal depends on the number of individual motor units that can be activated voluntary or reflexly in the patient. Disease processes that affect motor neurons or axons (*in neuropathy*) will reduce the number of available motor units for recruitment. On the other hand, the size and shape of the potential of any motor unit that remains functional are unaffected. The neuropathic processes result in denervation of the muscle fibers, and will produce hypersensitivity and fibrillations of affected fibers. Some weeks later, the remaining motor units will be larger than normal due to regeneration. *In myopathies*, only the individual muscle fibers are affected but not the size of motor units. Therefore the number of recruitable motor units and fibers doesn't change, but the motor units are weaker, because they have fewer functional muscle fibers than normal.

Because EMG is related to the electrical and not to the mechanical activity of the muscle, there is often a poor correlation only between EMG activity and the generated force. Therefore, in clinical electromyography, beside the EMG signal the exerted force is also monitored by dynamometry (dino = power, meter = measure).

7.3. Measuring motor unit recruitment and muscle fatigue

Aim of the practical: to observe, record and correlate motor unit recruitment with increased power of skeletal muscle contraction. During the practical, the activity of forearm muscles is measured by dynamometry and electromyography and differences between the two arms are also tested.

Used materials: EMG electrodes, lead set (SS2L), dynamometer (SS25L), Biopac M30/35/36 acquisition unit

Carrying out the measurements / experimental methods:

The disposable electrodes should be attached to each forearm of the investigated subject as shown in Figure 7.3. For optimal electrode adhesion, place the electrodes on the skin at least 5 minutes before the start of the measurement. The SS2L lead set should be attached to the electrodes of the dominant forearm, following the color code of Figure 7.3.A. (If the subject is right-handed, the right arm is generally dominant. In the case of left-handed persons, the left forearm is dominant.)

Make sure, that the Biopac MP30/35/36 acquisition unit is OFF! If is not, turn it OFF! Connect the cables to the device: the lead set should be plugged in the first channel (CH1) and the hand dynamometer to the second channel (CH2), as shown in Figure 7.3.B. Turn on the Biopac unit!
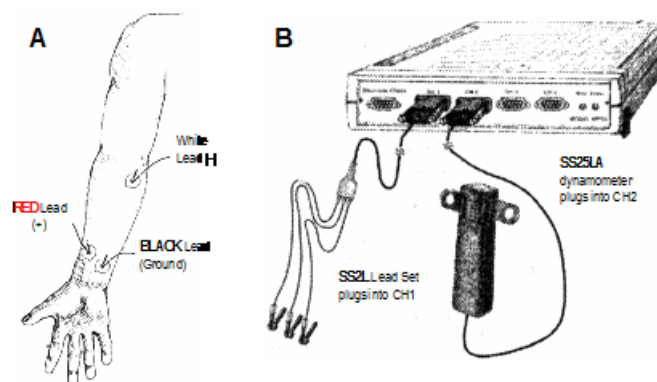
Figure 7.3. *(A) Positions of the electrodes on the forearm and the attachment of the lead set (SS2L) (B) connection of the lead set and the hand dynamometer to the Biopac device (B).*

To start the measurement, run the BSL 3.7.6 program and select the second lesson (Lesson 2: EMG II) from the pop-up list. At the beginning of data acquisition, a calibration procedure sets the internal parameters of the hardware (such as gain, offset and scaling), therefore it is critical for optimum performance. After clicking on the **[Calibrate]** button, follow the instruction of the pop-up windows: i) hold the hand dynamometer in your dominant hand, but release your grip (this establishes a zero force calibration). ii) after 2 seconds, clench it with maximum strength for 1-2 seconds. If the calibration procedure fails, it can be repeated by **[Redo calibration]** command. If the calibration was correct, the measurement can be started with the **[Continue]** command.

Experimental tasks

1. Study of motor unit recruitment: Measurements on the dominant arm are carried out first. After clicking the **[Record]** button, the program displays your clench force on the hand dynamometer channel. Repeat a cycle of Clench-Wait-Release-Wait on the dynamometer. Your signal on the screen should correspond to 5-10-15-20-25-30-40-50 kg. During the measurement, keep your clench on the dynamometer for 2 seconds and after you released it, wait at least 2 seconds! The cycles should be repeated until the maximum clench force is obtained. Press the **[Suspend]** button at the end of the segment! If the recording was not satisfactory, click on the **Redo** and repeat the segment!

2. Study of fatigue: By pressing the **[Continue / Resume]** button the recording will continue. Clench the dynamometer at a maximum force and try to maintain it as long as the displayed force on the screen is decreased by more than 50% of the maximum amplitude. If the measurement was correct, your data should look similar to Figure 8.4. By pressing the **[Suspend]** button the recording can be paused. If the recording was not satisfactory, click on the [**Redo**] and repeat the segment!

If the recorded data of the dominant arm is appropriate, attach the electrode leads to the other arm and press the **[Continue / Resume]** button to **repeat Task1 and Task2 on the non-dominant forearm.** Press the **[Suspend]** button between the segments! If you are finished with both forearm recordings press the **STOP** button!

Analysis

The recorded file can be opened from the Lessons menu with the **Review saved Data** command. Start the analysis on the data of the dominant forearm (section 1). Use the I-Beam cursor ( ) to select an area on the plateau phase of the clenches as shown on Figure 7.4. The selected area is highlighted with inverse color. Select the channel to be analyzed and the parameter to be measured. The value of the parameter (type) measured in the selected area will appear in the ***Measurement*** box. To do this, first you have to Calculate mean for each clenches on both channel 41 (*Clench Force*) and 40 (*Integrated EMG*)! Copy the values to the Journal by pressing **Ctrl+M**! Repeat the analysis on the data of non-dominant forearm (section 3).
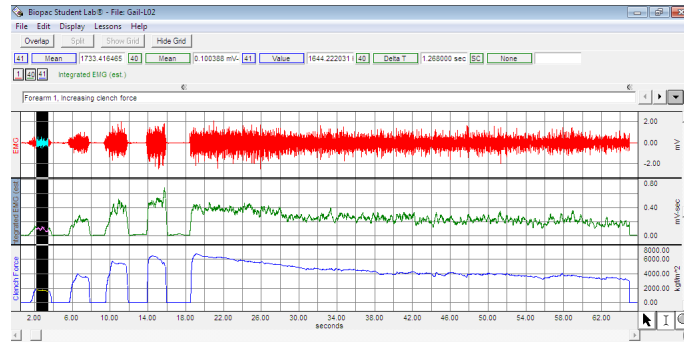
Figure 7.4. *Plateau of first clench selected in the case of properly performed measurement of dominant arm. Values for each channel can be read by using the I-Beam selection tool.*

The fatigue time should be calculated by using the DeltaT measurement type on channel 41 (measurement box 1). Set up the second measurement box to the value on channel 41! Use the I-Beam cursor to select a point of maximal (100%) clench force immediately following the start of the Task2 segments. Move the cursor to the right and find the point of 50% maximum clench force and leave the cursor there. Select the area from this point back to the point of maximal clench force.

Data report

- In the Data Report data from the dominant and the non-dominant arm of 2 different subjects (e.g. male - female or athletic - none sporty person) should be compared.

- Calculate strength – Integrated EMG ratio in every case!

- Plot integrated EMG data of subject's stronger and weaker arm in the function of muscle force on a common graph. Interpret the observed correlations and show that the phenomenon of recruitment is reflected in the results!

- Analyze the relationship of the EMG signal and the exerted force at the beginning of clench and at the final stages of the fatigue!

- Compare the fatigue time of forearms of each subject!

Chapter 8. Endocrinological functions

8.1. Introduction

Communication between cells and tissues of the organism often occurs via different chemical substances. Molecules secreted by certain cells bind to specific receptors and thus exert specific effects. Considering the "pathway" which the molecule uses to reach its receptor, we may distinguish autocrine, paracrine and endocrine signalling types (Figure. 8.1). In case of the latter, the signalling molecules (peptides, steroids, amino acid derivatives) are produced in specific hormone producing organs, called *endocrine glands*, and they are secreted into the bloodstream which carries them to their target cells. The cell secreting the hormone may be a neuron, in this specific case, we speak of neuroendocrine signalling. The effect of hormones depends also on the receptors; the same hormone may have different effects on different organs due to the differences in receptor type. Receptors bind hormones reversibly, with high affinity.
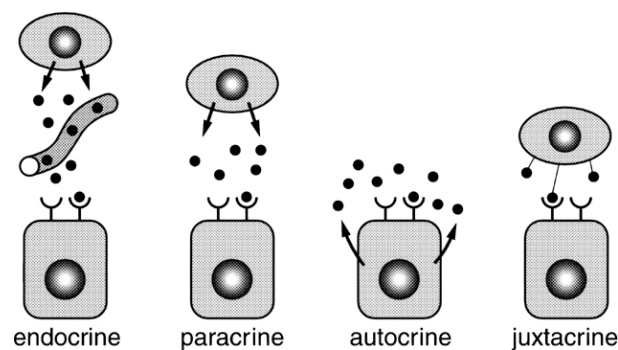
Figure 8.1. *The secretion of chemical signalling molecules, the localization of receptors and the different signalling pathway types are shown. In case of autocrine signalling, the receptors are located on the secreting cell itself. In case of paracrine signalling, the receptors are located on neighbouring cells (tissue hormones). Endocrine glands secrete their products into the bloodstream.*

The main regulatory organ of the endocrine system is the hypothalamus-pituitary gland complex (Figure. 8.2). The pituitary gland or hypophysis is located at the base of the brain, resting in a small cavity of the cranium (sella turcica). It is composed of an anterior lobe originating from the pharyngeal ectoderm, the *anterior pituitary or adenohypophysis*, and a posterior lobe of neuroectodermal origin, the *posterior pituitary or neurohypophysis*. A well developed intermediate lobe is present in many animal groups such as amphibians, but this is rudimentary in mammals. In the anterior pituitary several hormones are produced: TSH – thyroid-stimulating hormone, ACTH – adrenocorticotropic hormone, LH – luteinizing hormone, FSH – follicle-stimulating hormone, GH – growth hormone, PRL – prolactin. The latter two affect organs directly, while the rest of them act on other endocrine glands. The intermediate lobe produces MSH from pro-opiomelanocortin (POMC) that is also the precursor of ACTH and endogenous analgetic peptides. The posterior pituitary stocks oxytocin and antidiuretic hormone (ADH or vasopressin) produced in the hypothalamus; so these hormones are released from (but not produced by!) the posterior pituitary.
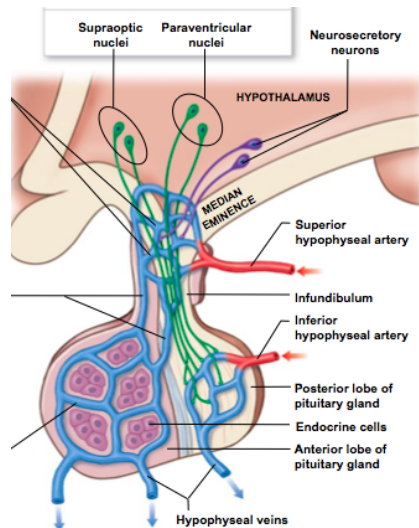
Figure 8.2. Schematic representation of the hypothalamus-pituitary system.

The endocrine glands regulated by the hypothalamus-pituitary system are the thyroid gland, the middle layer of the adrenal cortex and the sexual glands. Their functioning is regulated via negative feedback; this means that increase of the end product concentration (the hormone produced by the stimulated endocrine gland) inhibits the production of tropic (stimulating) hormones both in the hypothalamus and the hypophysis.

There are other hormone-producing organs/cell groups which are not under the control of the hypothalamus-pituitary system. Such are, for example, the Langerhans-islets of the pancreas, where insulin is produced; the parathyroid producing parathormon; or the placenta which secretes human chorionic gonadotropin and many other hormones.

8.2. Pregnancy tests

Investigating the effect of chorionic gonadotropin

During pregnancy, the blood concentration of gonadotropic hormones increases significantly, and thus their concentration in the urine rises also. This gonadotropic hormone does not originate from the adenohypophysis and there are major species differences in its structure. The hormone produced by the human placenta and appearing in the urine of pregnant women is called human chorionic gonadotropin (hCG). The hCG has primarily an LH-like effect. The high level of hCG in the urine makes it suitable for pregnancy tests. Previously applied biological tests are less sensitive than the immunological rapid assays used nowadays. They give a reliable result only in a later phase of pregnancy, compared to the modern tests, because they require a quite high urine hormone level.

8.2.1. Rapid immunological assay determining human chorionic gonadotropin (hCG)

Immunological tests are very sensitive and suitable to detect very early pregnancy. They do not require experimental animals and the result can be read within a few minutes. All types are based on the detection of hCG (present in the urine of preganent women) with anti-hCG antibodies recognizing the hormone. A drop of urine should be applied onto the sample spot of the strip. This creates a liquid front moving in the assay strip towards the result windows. Near the sample spot, a mobile (soluble) form of anti-hCG antibody is present in the strip (Ab1). The hCG-Ab1 complex and the free Ab1 will move with the liquid front towards the region of the result window containing immobile anti-hCG (Ab2). Ab2 can bind to a different part (epitope) of hCG than Ab1. If binding occurs, Ab2 triggers an enzymatic reaction and the insoluble product forms a visible coloured line.
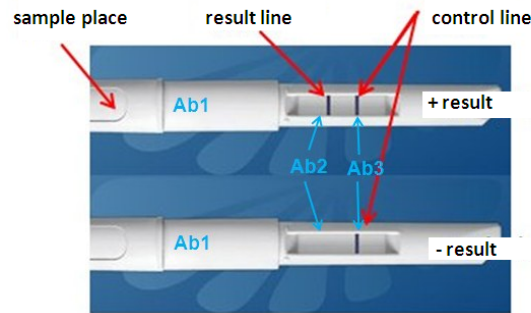
Figure 8.3. *Possible outcomes of a pregnancy test.*

The liquid front moves further and reaches a region where another immobile antibody is bound: Ab3 recognizing Ab1. When binding occurs here, this also triggers an enzymatic reaction leading to the appearance of a coloured line. The presence of this line proves that the urine sample moved all the way along the assay strip.

8.3. Determining blood glucose level

Insulin and *glucagon* are pancreatic hormones that regulate blood glucose level. In healthy subjects, food intake leads to elevated blood glucose level which increases insulin secretion. Once food is digested and glucose is absorbed, insulin secretion ceases, its level returns to the normal, pre-meal level. Insulin acts primarily by accelerating facilitated glucose uptake into muscle and adipose tissue cells and speeding conversion of glucose into glycogen, thus it reduces blood glucose level. Glucagon, on the other hand, antagonizes insulin-mediated actions and increases blood glucose level. Production of insulin and glucagon changes in opposite directions, and blood glucose level changes accordingly in pre-meal, consummation, post-meal, and starving periods.

Diabetes mellitus is one of the most common endocrine disorders and is diagnosed when pre-meal blood glucose level is over 7 mmol/l and is increased over 11 mmol/l values after a meal. In this case, insulin is unable to help glucose uptake by cells, thus excess glucose is excreted in the urine (glucosuria) leading to osmotic diuresis. Therefore, early hallmarks of diabetes mellitus are polyuria (excessive urine production), polydipsia (excessive thirst) and polyphagia (excessive eating).

There are two types of diabetes mellitus. In **Type 1 diabetes**, the level of endogenous insulin is diminished due to the decay of pancreatic beta cells. This form of illness develops mostly during at a young age, and is probably due to autoimmune reactions. Despite high blood glucose levels, cells are „starving" which can lead to coma and often to ketoacidosis. **Type 2 diabetes** is more common and normally occurs in obese people over the age of 35. Consummation of food with high glycaemic index (GI), i.e. food that increases blood glucose level quickly, stimulates increased insulin secretion. When this situation is prolonged and occurs very often, cells might lose insulin sensitivity because of the downregulation of the insulin receptors and thus, insulin resistance developes. Persistent hyperglycemia (increased blood glucose level) causes potentially serious damage to the blood vessels that supply nutrients to areas like eyes, kidneys, nerves and heart.

A blood glucose test measures the amount of glucose in your blood. Glucose comes from food containing carbohydrates and it is the main source of energy used by the body. In case of healthy individuals, pre-meal blood glucose level is between 3.5 - 5.5 mmol/l. When the level of blood glucose decreases, hunger and seeking for food is induced. Following carbohydrate consummation, blood glucose level increases fast, but its level returns below 8.0 mmol/l within 2 hours in healthy subjects.

8.3.1. Automated blood glucose meters (glucometers)

A glucose meter (or glucometer) is a medical device that can measure the approximate concentration of glucose in the blood (Figure. 8.4). It is a key element of home blood glucose monitoring (HBGM) by people with diabetes mellitus or hypoglycemia. A small drop of blood, obtained by pricking the skin with a lancet (fingerpricker), is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level. Test strips normally contain chemicals that react with glucose – it is a small spot impregnated with glucose oxidase and other components,

where the drop of blood is to be dropped. The chemical reaction induces optical changes which are measured by the reflected light in the glucometer. Each strip can be used only once.

As a rough estimation of blood glucose level, colour changes at the back of the strips can be also detected by eye and can be related to the coloured patterns displayed on the storage vial of the test strips. The instrument also displays the measured blood glucose level on its screen, normally in a mmol/l format. It is important to note that glucose levels in plasma are generally 10%–15% higher than glucose measurements in whole blood (and even more after eating). This is important because home blood glucose meters measure the glucose in whole blood while most lab tests measure the glucose in plasma.

8.3.2. Calibration of blood glucose meters

It is important that each type of glucometer has to be used with its own test strip! Since test strips may vary from batch to batch, some models require the user to manually enter a code found on the vial of test strips or on the test strips themselves. By entering the code into the glucose meter, the meter will be calibrated to that batch of test strips.

In case of using the *D cont personal* glucose meter, follow these steps:

1. Switch on the glucose meter by pressing the On/Off button.

2. Press button C(↓) to display the CODE signal on the screen and the actual saved code number.

3. To increase the already set code number, press M(↑), to decrease it, press C(↓). When the button is held down, numbers can be changed faster.

4. Once the desired code number is reached, press the On/Off button. This way the newly set code is stored and the glucose meter is ready to be used.

In case of using the *Accu Chek* glucose meter, the code chip found within each vial has to be inserted into the slot on the side of the device, when a new vial is opened.

8.3.3. How to use blood glucose meters

Switch on and calibrate the instruments accordingly to the above instructions. Insert an appropriate test strip into its slot – it has to be pushed in until it is securely positioned. The arrows on the strip should be upwards and point forward – it means that the coloured spot containing the reagent is also facing upwards. Disinfect and prick a finger according to previous instructions. After a few seconds, a blinking symbol of a drop of blood appears on the screen – **blood should be dropped onto the reagent spot while the signal is still blinking!**

In case of too low (below 1.1 mmol/l) or too high (above 25.5 mmol/l) values, the instrument displays **"Lo"** or **"Hi"** signal on the screen, respectively. Blood glucose level can be displayed in two formats: either in mmol/L or in mg/dl. In Hungary, the first format is used in the clinics. (To convert mmol/l to mg/dl, result is to be multiplied by 18. To convert mg/dl to mmol/l, divide the displayed value by 18.) The instrument can toggle between the both types of measurements; this can be set in advance by the small switch at the back.

8.3.4. Tasks to be carried out

Determine your blood glucose level! It is preferable to carry out the measurement twice: once in a pre-meal state (at least 5 hours later than the last meal) and once after consuming some carbohydrate-containing food or drink. In the latter case, wait at least 30 minutes between eating and the measurement. In healthy individuals, blood glucose level returns to normal, pre-meal values within 1-2 hours.

Figure 8.4. *The D Cont personal and Accu check blood glucose meters and the corresponding test strips.*

Write down the displayed values and explain the circumstances as the measurement was carried out. Compare the displayed values with reference values and discuss if any difference is observed.

Chapter 9. Investigating human perception – physical and physiological tests

9.1. Introduction

The most important role of the nervous system is to detect information from the environment and from the body, to process these stimuli and to elicit adequate - motor or hormonal - response reactions. The nervous and endocrine system maintains a stable internal environment (homeostasis) of the organism despite the continually changing environment.

The status and changes of inner and outer environments are detected by receptor cells which, in several cases, are grouped in **sensory organs**. The environmental effects interacting with our sensory organs are called stimuli. The **stimulus** excites the receptor cell (reception), leading to a change in the membrane potential of the **receptor cell** (*receptor or generator potential*). Receptor cells are either of epithelial origin, synaptically connected to the primary sensory neuron or it is the neuron itself which detects the stimulus (Figure. 9.1).
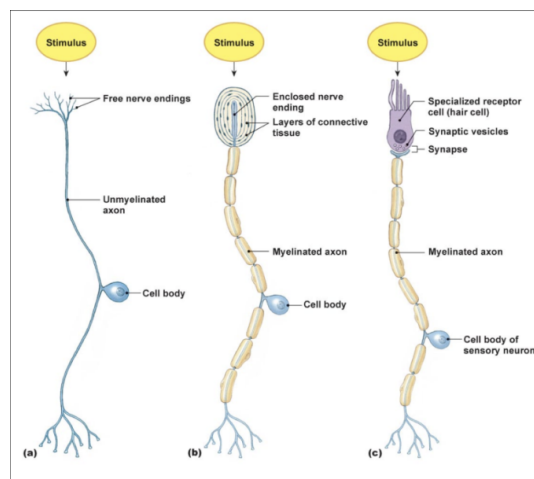
Figure 9.1. Possible relationship of receptor cells and primary sensory neurons. In most cases, the stimulus is detected by a modified process of the primary sensory neuron (skin receptors, muscle spindle, olfactory epithelium, etc.). Hair cells of the inner ear and gustatory receptors are cells of epithelial origin, synaptically connected to primary sensory neurons. Retinal cones and rods are connected to bipolar cells (which correspond to the primary sensory neuron), which in turn are connected to ganglion cells that are part of the CNS.

Information is transmitted from the sensory organs to the central nervous system by primary sensory neurons. Concerning their morphology, these are bipolar or pseudounipolar neurons, with their cell bodies located always outside the central nervous system (e.g. dorsal root ganglions), their axons form *afferent* pathways. The action potential is generated at the end of the peripheral process of the neuron, at the receptor site (if the receptor potential reaches the necessary threshold for action potential generation).

The first step of information processing in the central nervous system is **reception**, it results is **sensation**, and if it becomes conscious, **perception**. In order to be consciously perceived, the information has to reach the primary sensory area of the neocortex (for example, light information has to reach the primary visual cortex). **Sensory pathways** are neural pathways leading from the receptor to the cortical primary sensory area. The primary sensory neuron never reaches the cortex directly; it is relayed at least once, but usually twice. At the relay levels, the pre-liminary processing of the information begins; moreover, higher order centres may modify the functioning of lower centres (for example, receptor sensitivity may be modified).

Perception is a cognitive process following the primary processing of sensory information; it means the recognition and interpretation of the sensations. This process is significantly influenced by our previous experience (*memory*), by our *expectations* defined by our actual mood and motivations and even by the cultural environment that we live in. This explains how our sensations might be misinterpreted, leading to *illusions*.

It is not the characteristics of the stimulus that defines the psychic quality (subjective experience) and the *modality* of the sensation/percept, but the information-processing neuronal circuits of the central nervous system. (For example, a strong mechanical stimulus on the receptor cells of the retina leads to the sensation of light – we are seeing stars). We do not know the exact number of sensory modalities, but the classical senses have been known since the antiquity.

Tactile sensation is the detection of mechanical stimuli affecting the skin, thermosensation means the detection of thermal energy, while gustatory and olfactory processes mean the detection of dissolved or gaseous chemical substances. Pain sensation is evoked by tissue lesion – independently of the quality of the noxious stimulus. Vision (sight) is the sensation of electromagnetic waves, while hearing is the detection of mechanical vibrations of the environment.

Detection of sensations from inside of our body (interoception) is usually unconscious; it is in close connection with the vegetative nervous system (e.g. blood pressure receptors) or the regulation of movement (muscle spindles, tendon receptors, semicircular canals).

During the practical concerning perception, we will carry out experiments illustrating basic phenomena concerning human *hearing, vision, tactile- and thermosensation*. We will also examine the relationship of physical stimulus parameters and the evoked sensation; this relationship is the subject of *psychophysics*.

The ***absolute sensory threshold*** is the lowest intensity of a stimulus (e.g. sound intensity in decibel) at which the stimulus (sound) can be detected. The ***differential sensory threshold*** is the lowest difference between two values of the same stimulus parameter which can be distinguished. The value of both types of thresholds may change; they depend on the actual status of the sensory organs and of the central nervous system (e.g. attention level, arousal level), on the effect of previous stimuli (e.g. in case of the method of limits, the threshold value is different with ascending or descending stimulus sequences), etc. Therefore, the sensory threshold is a statistical concept; its value can be estimated based on several measurements. The ***maximal stimulus*** is the intensity level at which increasing the intensity does not elicit any further increase in sensation. The range between the threshold value and the maximal stimulus is called the ***sensory range***.

One method to determine the absolute threshold is the *method of limits*. In the first step, patient is stimulated by a sequence of stimuli that are first strong and easily detectable, but are continuously decreasing in intensity (*descending sequence*) until the subject is unable to detect them. Aftger that, another stimulation sequence is applied called *ascending sequence*. In this sequence, stimulus intensity increases from subthreshold to easily detectable. Both sequences should be repeated several times. This process yields several momentary threshold values. Then, mean values for ascending and descending sequences are determined separately. The mean value will usually be lower for descending than for the ascending sequences. The average of the means for the ascending and descending series will be considered as the absolute threshold.

A quicker and more efficient type of the above-mentioned method is the *adaptive method*. It is more efficient because, the intensity of *each new stimulus depends on the response of the patient*. The stimulation starts with a *suprathreshold stimulus*, then the intensity of the stimulus is *decreased* in predefined steps until the stimulus becomes subthreshold. The sequence is not ended here, like in the case of the method of limits. Stimulus strength is increased again until the subject can detect the stimulus again. The sequences of the decreasing and increasing stimuli are continued but the step size is halved at each turn. After reaching the previously defined minimal step size, the sequence is continued until the stimulation strength alternates several times between two values: the average of these two values will represent the threshold. (The value of the minimal step depends on the desired accuracy, the available time and the technical facililties.)

The *method of constant stimuli* may be also used for the determination of the absolute threshold. In this case, stimuli of varying intensities are presented in random order to the subject. The range of intensities goes from the surely subthreshold to the surely suprathreshold. The random sequences are given several times (with the same intensities, but in a different order). Then, for each stimulus intensity, the percentage of those cases in which the stimulus was detected is determined. The stimulus strength which was *percieved in 50 percent of the presentations* is defined as threshold.

For the determination of the differential threshold, all of the above-mentioned methods may be used, but stimuli should be presented in pairs, and the subject has to detect the difference between the test stimulus with varying strengths or frequency and the stimulus with constant strength and frequency (sample).

In contrast to the previous practicals, in this case, our measured data and conclusions concerning the sensation-perception experience will *depend on the report of the subject*. This subjectivity may lead to special measuring errors. If the characteristic error sources linked to human observation are known and controlled, then the data derived from several subjects will demonstrate objectively describable laws and principles.

9.2. Investigations of hearing

Sound is a mechanical wave: an oscillation of pressure transmitted through a solid, liquid, or gas medium at frequencies within the range of hearing. (Sound cannot travel through a vacuum.) **Hearing** (or **audition)** is the ability to perceive sound through an organ such as the ear. The speed of sound depends on the medium the waves pass through, the temperature and some other factors. For example, in the air at 20°C the speed of sound is approximately 343 m/s, in water about 1482 m/s.

Sound waves are longitudinal waves of alternating pressure deviations from the equilibrium pressure, causing local regions of compression and rarefaction. Pressure changes in time can be described by a sinusoidal function which is characterized by two generic properties: frequency and amplitude. Frequency is the rate of pressure variations caused by the sound; amplitude is the magnitude of pressure variations relative to atmospheric pressure.

As the human ear can detect a wide range of sound amplitudes, sound pressure is often measured on a logarithmic reference scale. The **sound pressure level** (SPL) is defined as: SPL (in **decibel** units) $=10 \log (P/P_0) = 10 \log (A/A_0)^2 = 20 \log (A/A_0)$, where P is the actual sound intensity or power, and P_0 is the reference intensity. The power or intensity (P) carried by a traveling wave is proportional to the square of the amplitude (A) or pressure.

The reference is generally the standard **threshold of hearing** at 1000 Hz of the human ear: 20μPa. Thus, the sound pressure level at this amplitude: SPL= 20 log (20μPa/20μPa) = 20 log (1) = 0 dB. Because of the great sensitivity of human hearing, the threshold of hearing corresponds to a pressure variation less than a billionth of atmospheric pressure!

A change in the pressure ratio by a factor of 10 is a 20 dB change: SPL=20 log (10) = 20dB.

A change in the pressure ratio by a factor of 10^6 is a 120 dB change. SPL=20 log (10^6) = 120dB.

The pressure at which sound becomes painful is the *pain threshold pressure*. It is about 120 dB. Pain threshold is a subjective category, which varies only slightly with the frequency. The **just noticeable difference** in sound intensity for the normal human ear is about 1 dB.

The ear has external, middle, and inner portions. The outer ear includes the pinna (or auricle), the ear canal, and the ear drum (or tympanic membrane). Pinnae capture the sound waves (the bigger the pinna, the more the sound energy it captures). The auditory canal acts as a closed tube resonator, enhancing sounds most effectively in the frequency range of human voice (2-5 kHz).

The middle ear is an air-filled cavity, includes the three ossicles (or ear bones): the malleus (or hammer), incus (or anvil), and stapes (or stirrup). The malleus is attached to the eardrum. The incus is the bridge between the malleus and stapes. The stapes' footplate is on the oval window of the inner ear. The eardrum and the ear bones ensure the **lossless coupling** between vibration of the air and vibration of the fluid in the inner ear. Without this arrangement a significant part of the sound would be reflected from the air/water boundary because of the great difference of their impedance.

Moreover, there are several simple mechanisms in the middle ear that combine to even increase the sound pressure. The first is the "hydraulic principle": The surface area of the tympanic membrane is many times larger than that of the stapes footplate. Sound energy that strikes the tympanic membrane is concentrated to the smaller footplate, giving an amplification of about ten. A second mechanism is the "lever principle". The ossicles can be thought of as a compound lever which achieves a multiplication of force by a factor of about two.

The inner ear includes both the organ of hearing (the cochlea) and the organ of equilibrium that senses both gravity and acceleration (labyrinth or vestibular apparatus). The cochlea is a spiral-shaped cavity in the bony labyrinth in which waves propagate from the base (next to the middle ear) to the apex (the top of the spiral) then back to the base. In humans it is 32-33mm long and makes 2.5 turns around its axis. The name is derived from the Latin for snail shell, in reference to its coiled shape. Within the cochlea are three fluid filled spaces: the scala tympani, the scala vestibuli and the scala media. The superior scala vestibuli (containing perilymph) starts with the oval window. At the apex of the cochlea it merges with the inferior scala tympani which terminates at the round window. The middle part is the scala media (containing endolymph), or cochlear duct separated by Reissner's membrane from the scala vestibuli and by the basilar membrane from the scala tympani.

The organ of Corti – located on the basilar membrane - is made up by a single row of inner hair cells, three rows of outer hair cells and pillar cells supporting the hair cells. Outer hair cells are acoustical pre-amplifiers and have evolved only in mammals. Damage to these hair cells results in decreased hearing sensitivity.

The organ of the sound perception is in the middle ear. Tthe energy of pressure waves is translated into mechanical vibrations. The cochlea propagates these as fluid waves which in turn put basal membrane to motion. This causes deflections of the hair-cell stereocilia and opens mechanically gated ion channels. The influx of positive ions depolarizes the cell, resulting in a receptor potential. This receptor potential triggers the release of neurotransmitters (glutamate) at the basal end of the cell. The neurotransmitter released by hair cells stimulates the neurons of the auditory nerve (VIIIth cranial nerve). The sound information, now encoded as nerve impulses travels through many intermediate stations (such as the cochlear nuclei, superior olivary complex, inferior colliculus and lateral geniculate nucleus) and eventually reaches the primary auditory cortex, which is located in the temporal lobe.

The basilar membrane of the inner ear plays a critical role in the perception of pitch according to the place theory (tonotopy). Georg von Bekesy (1899-1972) found that movement of the basilar membrane resembles that of a traveling wave; the shape of which varies based on the frequency of the pitch. In response to low frequency sounds, the wide and loose tip of the membrane moves the most, while in case of high frequency sounds, the narrow and tight base of the membrane moves the most.

The perception of sound in any organism is limited to a certain range of frequencies. For humans, hearing is normally limited to frequencies between about 16 Hz and 20,000 Hz. The upper limit generally decreases with age (presbycusis). Other species have a different range of hearing. For example, dogs can perceive vibrations higher than 20 kHz, but cannot sense sounds below 40 Hz.
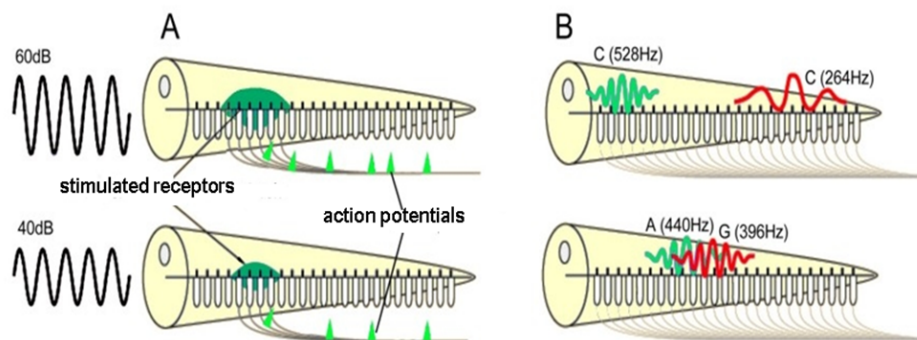
Figure 9.2. *Function of the Organ of Corti. A: Stimulus intensity is coded by frequency code (firing rate increases with increasing stimulus intensity) and population code (increasing stimulus intensity activities more neurons). B above: The place theory. In low frequency sounds the tip of the membrane moves the most, while in high frequency sounds, the base of the membrane moves most. B below: If two sounds are widely separated in pitch, their loudness is summed, but if they are in each other's critical band, they do compete for the same nerve endings on the basal membrane, and the "rule of thumb" for loudness is applicable.*

In most sensory systems – including auditory system - stimulus intensity is coded by frequency code (firing rate increases with increasing stimulus intensity) and population code (increasing stimulus intensity activities more neurons). **Loudness** is a subjective measure of sound intensity often confused with the objective sound pressure level (in decibels). Loudness is a psychological correlate of physical strength of the sound. The correlation is not

linear: at any given frequency, a general "rule of thumb" for loudness applies, stating that the power must be increased by about a factor of ten to sound twice as loud.

However, loudness is also affected by parameters other than sound intensity. The logarithmic rule for loudness is applicable only if the subject is listening to one sound. If a second sound is given that is widely enough separated in frequency from the first – in other words, it is outside the so called **critical band** - then this rule does not apply at all, and the subject will feel the sum of the two sound intensities. The explanation for this phenomenon comes from the place theory of pitch perception. If the second sound is widely separated in pitch from the first, then the sounds do not compete for the same hair cells and nerve endings. Nerve cells have maximum rates at which they can fire - called saturation (Figure. 9.2B).

The sensitivity of the human ear changes as a function of frequency. Humans with good hearing are the most sensitive to sounds around 2–4 kHz, then the thresholds increase to either side of the audible frequency range finally reaching the threshold of pain (Figure. 9.3). The unit of loudness is phon. By definition, 1 phon is equal to 1 dB SPL at a frequency of 1 kHz. Each line on the equal-loudness graph shows the SPL required for frequencies to be perceived as equally loud (Figure. 9.3).
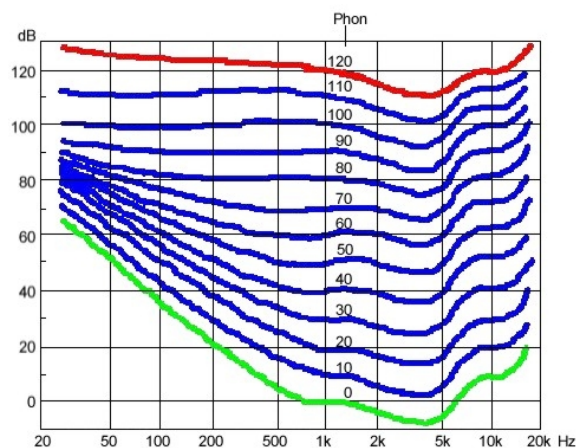
Figure 9.3. Sensitivity of the human ear as a function of frequency. Humans are the most sensitive to sounds around the frequency of human speech; the thresholds then increase on either side of the audible frequency range and finally meet the threshold of pain.

The human auditory system averages the effects of sound over a 600–1.000 ms interval. A short sound of constant SPL will be perceived to increase in loudness as its duration increases up to a duration of approximately 1 second at which point the perception of loudness will become constant.

The ear is sensitive to ratios of frequencies not only absolute frequencies (**pitches**). The musical frequency intervals that are generally the most consonant (pleasant) to the human ear are intervals represented by small integer ratios. (For example: the octave 2:1, fifth 3:2, and fourth 4:3.) The equally tempered scale is the common musical scale used at present. It divides the octave into 12 equal semitones, and each semitones into 100 cents. The just noticeable difference in pitch corresponds to about 5 cent (with great personal differences).

Natural sounds may be generally characterized not only by pitch and loudness, but also by quality. **Timbre** describes those characteristics of sound which allow the ear to distinguish sounds of different sources which have the same pitch and loudness. Timbre is mainly determined by the harmonic content of a sound and the dynamic characteristics of the sound such as vibrato and tremolo and the attack-decay envelope of the sound. For sustained tones, the most important of these is the harmonic content, the number and relative intensity of the upper harmonics present in the sound. The attack and decay is a rise and fall to/from the sound's peak amplitude. The vibrato is a periodic change in the pitch of the tone and the tremolo is a periodic change in the amplitude of the tone.

9.3. Experiments on the hearing system

Aim of the practical: To analyse detection of spatial sound source localization, and to compare air and bone conduction by Rinne tuning fork test.

Materials needed: PDD-401 audiometer, headphone, computer, phonendoscope, tube clamp, ruler, tuning fork.

9.3.1. Pure tone audiometry (PTA)

Audiometry is the science of measuring hearing acuity for variations in sound intensity and pitch. The most commonly used assessment of hearing is the determination of the threshold of audibility, i.e. the level of sound required to be just audible.

We use PDD-401 audiometer to identify hearing threshold levels. Audiometric test should be carried out in a room where the background noise is less than 18dB (external sounds influence the test.)

Steps of the experiment:

• Fit the headphones carefully over the ears! Start the **SpiroWin** software!

• Click on the button to open the patient database! Click on the button, and fill up the patient's datasheet! Don't forget to save it ()!

• Start the audiometry by clicking on the button (Figure. 9.4)!

• Choose automatic mode (), then click on button! (Test can be stopped any time by clicking on the button.) In automatic mode both of the subject's ears are tested through a range of frequencies (125, 250, 500 and 750Hz, 1, 1.5, 2, 3, 4, 6 and 8 kHz) at varying degrees of loudness in a decibel range between –10dB and +110dB. The subject is required to indicate whether he/she can just hear a certain sound by pushing the SPACE button on the keyboard. *Subject should not see the computer screen because it can influence the results!*

• The procedure is repeated until three identical threshold results of each frequency. From this data both a numerical and a graphic representation (an audiogram) of hearing abilities at various frequencies is produced.

• Save () and print () the result at the end of the experiment! Evaluate the audiogram!
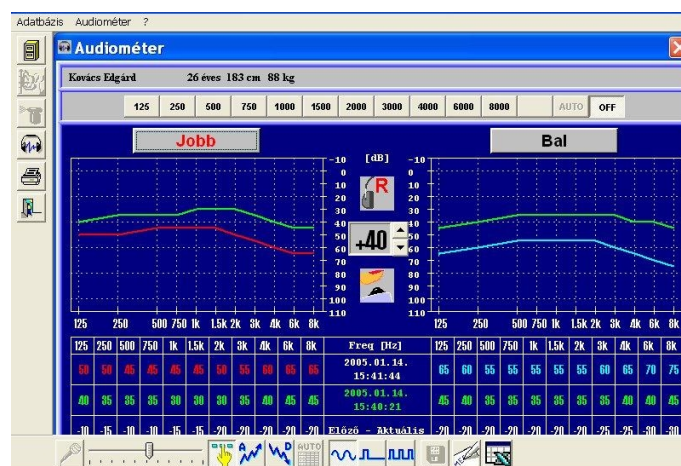
Figure 9.4. *SpiroWin software. Screen of audiometry*

9.3.2. Sound localization test

Sound localization refers to a listener's ability to identify the direction of the source of the detected sound. To determine direction, the auditory system analyzes information collected from both ears. Interaural time differences: sound from the right side reaches the right ear earlier than the left ear. Interaural level differences: sound from the right side has a higher SPL at the right ear than at the left ear, because the head shadows the left ear. For frequencies below 800Hz, the dimensions of the head are smaller than the wavelength of the sound waves. Therefore, the auditory system can determine phase delays between the signals detected by the left and right ears. (Interaural level differences are very low in this frequency range.) For frequencies above 1600Hz the length of the sound waves are too small to determine an interaural phase difference. However, the interaural SPL differences become larger, so mainly these differences are evaluated by the auditory system. If there is an abrupt sound onset, the delay of this onset between the ears also can be used. Between 800Hz and 1600Hz there is a transition zone, where both mechanisms play a role. Localization accuracy is 1 degree for sources in front of the listener and 15 degrees for sources to the sides.

Steps of the experiment:

Subject should put on the phonendoscope from behind (the bell should be at the back of the subject. Noise can be generated by carefully touching the membrane. Subject should show the determined direction of the sound by his/her hand.

- At first the two eartubes are at the same length, so the subject should hear the sound from the middle. (Note any differences!)

- Lengthen the adjustable eartube to produce phase- and onset- delay (and also an intensity loss because lengthening the tube means increase of source distance too). Note the direction detected by the subject!

- Produce intensity loss by reducing the tube diameter! (Use a tube clamp on the fixed eartube!) Note the detected source direction again!

- Leave the clamp on the fixed eartube and change the length of the adjustable one until the subject senses the sound from the median! Measure the length difference between the two eartubes!

9.3.3. Rinne tuning fork test

Air conduction uses the apparatus of the ear (pinna, eardrum and ossicles) to amplify and direct the sound to the inner ear. On the other hand, bone conduction bypasses these parts and allows the sound to be transmitted via the bones directly to the inner ear albeit at a reduced volume. In the case of outer source the bone conduction has minor role in the hearing process, because of the great loss of sound energy at the air/bone boundary. But the vibration of the speaker's larynx also vibrates the tissues of his/her head. This pathway tends to alter the quality of the sound sensed by the speaker. This is why our own speech recorded and played back sounds so different (Figure. 9.5).
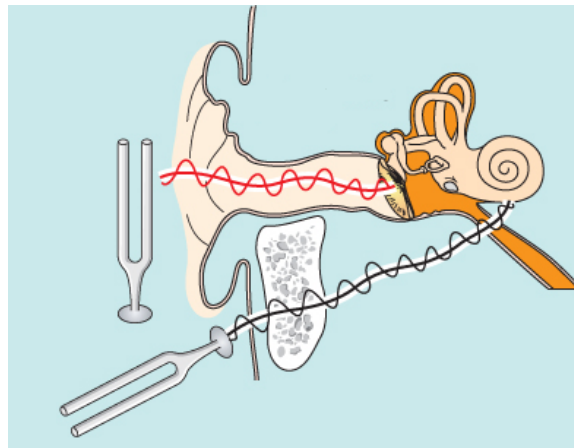
Figure 9.5. Steps and the pathways of Rinne's test

Steps of the experiment:

- Place a vibrating tuning fork (512Hz) on the top of the skull until sound is no longer heard by the subject.

- Then, place the fork immediately next to the ear! The subject should report when the sound caused by the vibration is no longer heard.

In the Rinne tuning fork test, air and bone conduction are compared. In normal hearing tones are louder by air conduction than by bone conduction. In conductive hearing loss (see below), however, the bone-conduction stimulus is perceived as louder. In sensorineural hearing loss both air and bone conduction sounds are diminished, but the air conduction sound is perceived as louder. So the Rinne tuning fork tests can distinguish between conductive and sensorineural hearing losses.

Conductive hearing loss occurs when sound waves are prevented from passing from the air to the fluid-filled inner ear. This may be caused by a variety of problems including buildup of earwax, infection, fluid in the middle ear, a punctured eardrum, or fixation of the ossicles, as in otosclerosis. Once the cause is found and removed or treated, hearing usually is restored.

Sensorineural hearing loss develops when the auditory nerve or hair cells in the inner ear are damaged. Sensorineural hearing loss frequently occurs as a result of the aging process in the form of presbycusis but can also be the result of repeated, continuous loud noise exposure. Tumors, viral infections or toxic medications can lead to sensorineural hearing losses. Generally, it is non-reversible.

Evaluate the subject's audiograms! Is there any difference in the thresholds of different frequencies from the normal values? How can you explain the difference? Analyze the effect of lengthening and constriction of the eartubes of the phonendoscope! Which degree of lengthening of the adjustable side of airtubes could compensate the constriction of the other? Evaluate the Rinne tuning fork test! Was there any difference between air and bone conduction by means of hearing sensitivity?

9.4. Sometosensory receptors

The **somatosensory system** includes several modalities of sensation from the body – touch, pressure, pain, temperature and proprioception. These modalities are transmitted to the central nervous system through three different pathways. The first modality is called **discriminative touch.** This means sensation of touch, pressure, and vibration by which one can describe the shape and texture of an object without seeing it. The second types of modalities are **pain and temperature** (and also itch and tickle). The third modality is the **somatosensory proprioception** - muscle stretch, tendon tension, etc.

Tactile stimuli are mechanical forces that get in contact with the skin. Touch is caused by a force that produces little distortion of the skin. Pressure means greater distortion of the skin and also the underlying tissue. Tactile stimuli varying in time produce sensations such as flutter or vibration.

The cell bodies of the somatosensory afferent neurons are located in the posterior root ganglia of the spinal cord. They are pseudounipolar cells with a single process that divides to form one peripheral and one central axon. The peripheral axon ends in the skin (muscle, etc.), and the central axon travels to the central nervous system. Most of the somatosensory afferent terminals are enveloped in a connective tissue capsule or end on hair follicles. The complex of the capsule/hair follicle and the afferent endings form a "somatosensory receptor". The sensitivity of the receptors to specific stimuli is determined by the non-neural tissue surrounding the afferent terminal (Table 9.1). Other somatosensory axons branch and terminate in skin as free nerve endings.

Table 9.1. Skin senses and receptors

Name	Shape	Adaptation	Localization	Sense
Merkel	Couple of modified epithelial cells (Merkel cell) innervated by specialized nerve ending branches (Merkel disk).	slow	in the basal layer of the epidermis	touch and form

Meissner	Egg shaped. Stack of flattened epithelial cells with afferent terminal fibers interdigitated between them.	fast	in a hairless skin, near the surface of the skin	flutter and movement
Krause	Ball shaped	slow	near the surface of the skin	flutter, and **cold**
Ruffini	Cigar-shaped. Longitudinal strands of collagenous fibers with nerve ending branches intertwined with them.	slow	deep in the skin	pressure, **warm**
Vater-Pacini:	Onion shaped. Concentrically layered epithelial cells with a single nerve ending in its center.	fast	in subcutaneous tissue beneath the dermis	vibration
nerve endings in hair follicles	Nerve endings spiral around or run parallel to the hair shaft	fast	in hair follicles	movement
free nerve endings		fast or slow	superficially	touch, pressure temperature, pain, itch etc.

The **receptive field** of a sensory neuron is a region of space in which the presence of a stimulus will alter the firing of that neuron. In the somatosensory system, receptive fields are regions of the skin. The fingers, which require the ability to detect fine detail, have many mechanoreceptors with small receptive fields, while the back and legs, for example, have fewer receptors with large receptive fields.

Thermoception or **thermoreception** is the sense by which an organism perceives the rate of heat flux. The organs of Ruffini are the heat receptors while the bulbs of Krause are the cold receptors. (Both are considered to be mechanoreceptors too, because they respond also to mechanical stimuli, e.g. vibration.) Other thermoreceptors are free nerve endings.

Cold receptors respond to lower temperatures (10°C -40°C), warm receptors are activated by heat (30°C -45°C). Warm receptors are the terminals of unmyelinated C-fibers (low conduction velocity), while those responding to cold belong to thinly myelinated Aδ fibers (faster conduction velocity). Some cold receptors also respond to sudden high temperatures (typically above 45°C), and some warm receptors respond to sudden cold temperatures (below 10°C). These are known as paradoxical cold and heat responses, respectively. Both might be followed by a sensation of pain. The neutral zone (between 32 to 35 °C) is defined as the range of temperatures in which heat and warm receptors are equally active and small adjustments of skin blood flow are sufficient to maintain core temperature. Long lasting cold stimuli block thermoreceptors – and other nerve fibers too – cooling is a well known anesthetic procedure. Localization of the heat (or cold-) source is better if it is coupled with tactile senses.

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Normally, pain motivates the individual to withdraw from damaging situations and to avoid similar events in the future, and also to protect damaged tissues during healing.

Nociception is initiated by nociceptors (pain receptors), that can detect mechanical, thermal or chemical changes above a given threshold. All nociceptors are free nerve endings. Nociceptors are found in the skin and on internal surfaces such as the periosteum or joint surfaces. A characteristic feature of nociceptors is their tendency to be sensitized by prolonged stimulation, leading to a condition called hyperalgesia.

Skin nociceptors may be divided into two categories. The first type includes the high threshold mechanical nociceptors responding only to painful mechanical stimulation, and the thermal nociceptors, which respond to extreme thermal stimuli (specific nociceptors). The second types are the polymodal nociceptors, which respond to all (mechanical, thermal, chemical) high intensity stimuli. Chemicals which can activate nociceptors include serotonine, histamine and bradikinin. Other chemical mediators, like prostaglandins act as sensitizers, altering pain sensitivity by increasing the responsiveness of peripheral nociceptors. Aspirin-like pain-killing drugs act by inhibiting prostaglandin synthesis (Figure. 9.6).
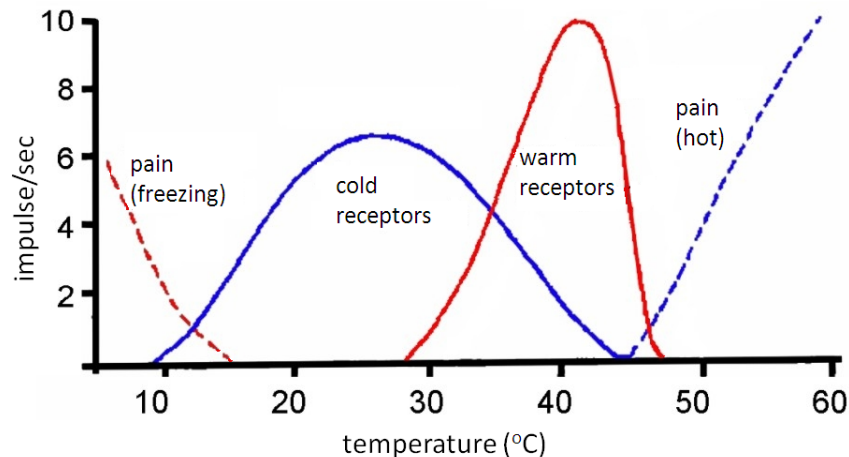
Figure 9.6. Sensitivity zones of the thermoreceptors

Primary sensory neurons involved in pain sensation release predominantly substance P and glutamate in the dorsal horn of the spinal cord. Nociceptive information is transmitted to the brain via the spinothalamic tracts. Noxious signals are sent to the reticular formation and thalamus and enter consciousness. From the thalamus, the signal travels to the somatosensory cortex, where the pain is experienced and localized. Nociception can also cause generalized autonomic responses before or without reaching consciousness to cause tachycardia, hypertension, pallor, cold sweating, nausea and fainting. Finally, the ascending information can activate descending analgesic pathways, from the midbrain periaqueductal grey area, which exerts an inhibitory control over the dorsal horn. Opioid receptors are present in many regions of the nervous system that are involved in pain control. The opioid drugs produce analgesia by inhibiting neurotransmitter release from the primary afferent terminals in the spinal cord, by inhibiting the projection neurons transmitting pain information or by activating the descending analgesic pathway in the midbrain.

Itch is „a sensation that causes the desire or reflex to scratch". Itch has many similarities to pain, but while both are unpleasant sensory experiences, their behavioral response patterns are different. Unmyelinated nerve fibers for itch and pain both originate in the skin, however, pain creates a withdrawal reflex while itch leads to a scratch reflex.

9.4.1. Examination of tactile receptors and thermoreceptors of the skin.

Aim of the practice: study the functioning of tactile and thermoreceptors of the skin

Materials needed: beakers, thermometer, compass, ruler

Thermoreceptor adaptation test

Prepare three beakers. First container marked „HOT" should be filled with 40 C° water (hot tap water, warmed up if needed). Second container marked „ROOM TEMPERATURE" should be filled with room-temperature water. Third container marked „COLD" should be filled with 10 C° water (cold tap water with ice cubes). Check the temperatures with a thermometer.

Steps of the experiment:

• Subject should place one finger in the COLD container and an other finger in the HOT container!

• Leave them in the water for 15 seconds! How do the fingers feel? Does it change over the course of 15 seconds? Does it feel warmer or colder than it did originally? Note any changes!

• Place both fingers in the ROOM TEMPERATURE container! How does the fingers feel? Warmer or colder?

The experiment demonstrates the adaptation of themoreceptors, and relativity of thermal sensation. Activity of cold receptors decreases in the cold water because of the long lasting cold stimuli. The same happens to the warm receptors in the warm water. Because perception of temperature depends on the relative activity of warm and cold receptors, the finger kept in hot water senses ROOM TEMPERATURE water colder then the finger kept in cold water.

Note every changes in temperature sensation during the whole experiment! Explain your results! Compare the adaptation time of cold vs. warm receptors. Find an explanation of this difference!

Two point touch discrimination test

The spatial resolution to touch can be evaluated by measuring two-point discrimination thresholds. The subject has to report whether his/her skin is touched with one or two pointy objects (e.g. a pair of compass with two spikes). If the two stimulated points belong to the same receptive field, the sensation is felt as one stimulus. If the points belong to separate receptive fields, the subject feels two stimuli. The distance between the points, at which the subject loses the ability to feel two points is the **diameter of the receptive field**. The two-point discrimination threshold is less than 5 mm at the finger tips and is about 40 mm at the thigh.

Steps of the experiment:

- The subject should close his/her eyes. Start with setting the compass spikes 2-3 cm apart! Touch to subject's skin with both spikes simultaneously. If he/she can feel two points, decrease slightly the distance and repeat! If he/she can feel one point, open the legs of the compass slightly and repeat!

- Find the shortest distance of compass spikes at which the subject still senses two touches. (Use a ruler to measure the distance!)

- Repeat this measurement in different regions of the body (index finger, palm, lower arm, back etc.).

9.5. Investigating vision - I. The optical system of the eye and the retina

Visible *light* is a frequency range of the electromagnetic radiation which evokes *light sensation* in the eye. For humans, the wavelength of visible light is between 380 nm and 780 nm. Vision is the detection and perception of these electromagnetic radiations. The elementary unity (quantum) of the energy radiation is called photon. Some optical phenomena are easier to explain if we consider the photon as a particle and the light as the current of these particles. Other phenomena are more easily explained if we consider the light as a wave. Sunlight contains waves with all the different wavelengths which make up visible light. These light waves with different wavelengths elicit different colour sensations in the eye.

A fraction of light reflected from the surface of different objects enters the eye via the pupil and reaches the retina; that is how objects are perceived. As the diameter of the pupil is quite large (particularly in week light), from each point of the object, several light rays of different direction reach the eye. For the creation of a clear image, these light rays have to be focussed in one single point, exactly on the retina. For this, optical lenses are needed in the eye, just like in a camera. **The optical system of the eye is composed by the cornea, the aqueous humour, the lens and the vitreous humour.** Because of its wave-nature, light is refracted at the interface of two media with different refractive indexes (in the case of the eye, most of the refraction occurs at the air-cornea border and at the two surfaces of the lens). The cornea, the lens and the vitreous humour behave as convex lenses because of their form. The relaxed eye has a refractive power of circa 60 diopters (i.e. its focus is 100/60 = 1.67 cm), considering the convexity and refractive index of its components. The optical system of the eye projects an inverted, reduced, real image of the outside world onto the retina. The image of the fixed object is projected exactly on the fovea centralis (Figure. 9.7).

Light can reach the receptor cells only across the pupil. The pigment layer forming the basis of the retina prevents light reflection and absorbs light coming from other directions (outside). So the eye functions as a real dark-room.
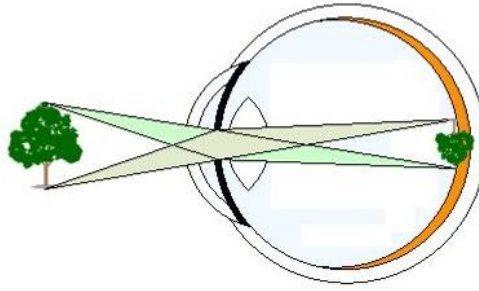
Figure 9.7. *Simplified scheme showing the projection by the optical system of the eye. The optical system of the eye projects an inverted, reduced, real image of the outside world onto the retina. From each point of an object, several light rays of different direction reach the pupil, but the optical system of the eye focuses the divergent rays into one point on the retina.*

The innermost layer of the eyeball is the **retina**, containing the light detecting receptor cells (the cones and rods), the primary sensory neurons (the bipolar cells), the ganglion cells that are considered as an outpost of the CNS, and several other cell types regularly distributed in ten layers. There are two special spots on the retina: the blind spot and the macula lutea (Figure. 9.8A). The macula lutea, more exactly the little depression in the middle of it, the **fovea centralis** is the spot for acute and detailed vision. This part of the retina contains no blood vessels; moreover the inner layers of the retina are displaced to allow the light pass directly to the cones (Figure. 9.8B). At the blind spot, the axons of the ganglion cells leave the eyeball, forming the optic nerve, and blood vessels supporting the retina enter and leave the eyeball here. This part of the retina does not contain any receptor cells.

The photoreceptor cells of the retina are **the rods and the cones**. The cones are responsible for photopic vision that is for colour vision in daylight. In the fovea, there are practically only cones, their quantity decreases dramatically towards the periphery of the retina (Figure. 9.8). The sensitivity of cones is suitable for daylight conditions: their stimulus threshold is quite high but they detect well the differences in luminosity under well-lit conditions. Cones may belong to three groups according to their photopigment type. The "green" cones (M-cones from medium wavelength) have an absorption maximum of circa 530 nm. Monochromatic light having this wave length is perceived as green. "Red" cones (L-cones from long wavelength) are most sensitive to light with a longer wavelength (560 nm), i.e. with yellow-red colour, while "blue" cones (S-cones from short wavelength) are most sensitive to light with a short wavelength (420 nm), i.e. with violet-bluish colour[1]. Our eye does not perceive different components (colours) of the spectrum as having the same "strength" even if they have the same energy level: the eye is most sensitive in the middle of the visible spectrum (550 nm, green colour), its sensitivity decreases towards the two extremities of the spectrum.

The rods are responsible for scotopic (twilight) vision. They can detect a single photon, but they become saturated already at medium light intensities, so their role in daytime vision is minimal. They are distributed mostly on the periphery of the retina. As all rods contain the same photopigment (rhodopsin), they cannot distinguish colours.
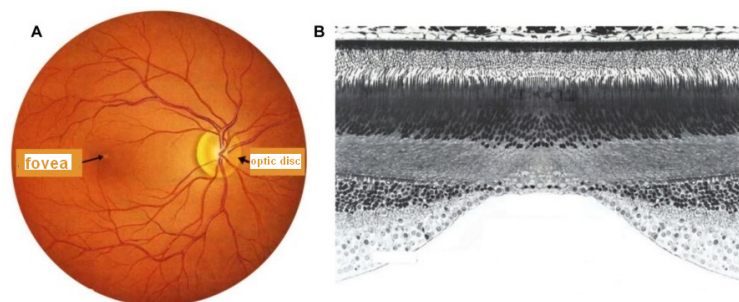
Figure 9.8. *The retina and its special spots. The blood vessels supplying the retina have a characteristic distribution. The convergence point of the blood vessels has a somewhat lighter yellow colour than the rest of the retina; this*

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[1]The colour of an object depends on which components of the white light are absorbed and which are reflected by it. If it reflects most of it, all three cone types are activated and we perceive white light. If it does not reflecfot any light, we see the object as black. If it absorbs blue and red light rays – like the chlorophyll pigments of the plants - , it is mostly green light that reaches our eye and activates the green cones. That is why we see plants as being green.

is the optic disc or blind spot. In temporal direction from the blind spot, we find a little darker spot, the macula lutea. The macula is closely surrounded by blood vessels, but does not contain any (A). Retinal structure near the macula. At the fovea, light is projected directly at the cones, because the inner layers of the retina are displaced (B).

Aims of the practical: Determine if the optical system of your eyes is perfect. Determine the resolution capacity and near point of your eyes. Map the field of vision of one of your eyes, identify the location of the blind spot and its distance from the macula. Check if your colour vision is correct. Observe the special spots on the retina.

Materials needed for the practice: artificial eye model, sclera lamp, perimeter, plates for testing colour vision, Snellen's charts, Placido's disc.

Detailed description of the practical:

9.5.1. Purkinje's blood vessel shadow experiment

There are two blood vessel systems supplying the retina. The arteries of the ciliary body (arteriae ciliares) enter the eye far from the optic nerve and, branching out, form a dense radiation, the choroid. This system supplies with oxygen and nutrients the lower layers of the retina which contain the photoreceptors. The central retinal artery (arteria centralis retinae) enters the eyeball in the middle of the optic nerve, forms four large branches and supplies the inner layers of the retina. These blood vessels are located in the superior layers of the retina. At the fovea, there are no blood vessels, but a particularly dense network of capillaries surrounds it.

Under normal conditions, we do not see the blood vessels of the retina, because our visual system quickly adapts to "images" which move together with the retina and are projected continuously to the same points of the retina. (If, with a special camera equipped with eye movement detectors, we project such a stabilized image on the retina, the image disappears in a few seconds.

Close your eye and touch the lower eyelid with the sclera lamp and move it slowly and gently while looking upwards (with closed eyes). This way, the light comes from an unusual direction and the shadows of the blood vessels can be seen. However, without continuously moving the sclera lamp, this image also quickly disappears. This experiment also proves that the photoreceptor cells of the eye form the lowermost layer in the retina (inverse eye), as the distance between the photoreceptors and the vessels on the surface of the retina is large enough only in that case to cause a considerable shift of the shadow of vessels shed on the photoreceptors when the direction of light changes.

9.5.2. Ophthalmoscopy on an artificial eye model.

Ophthalmoscopy is one of the most often used ophthalmological tests to check the status of the inner surface of the retina. Before the examination, the pupil is usually artificially dilated, to allow the observation of a bigger retinal surface. The method consists of projecting the light of a testing lamp via a mirror system into the eye of the patient, the light is then reflected into the doctor's eyes. The test is used to identify alterations of the refractive media located in front of the retina (clouding of the lens or the vitreous body); and at the retinal surface, to look for alterations of the blind spot, the macula and the blood vessels. Ophthalmoscopy is suggested in case of any eye complaint, but it is also carried out in case of diabetes, hypertonia, arteriosclerosis etc., because the eye is the only site in the body where the status of blood vessels may be directly studied. The alterations in retinal blood vessels indicate the status of the whole body's capillary network. Prognosis for the above-mentioned diseases may be drawn based on the ophthalmoscopy results.

As a strong light source may be dangerous in inexperienced hands, use only the artificial eye model! With this practical, we want to demonstrate the method used by doctors to examine the retina. Identify the blind spot and the macula on the model! Observe the larger blood vessels entering the eye at the blind spot! Prepare a schematic drawing of your findings!

9.5.3. Determination of visual acuity with Snellen's charts

When fixing to an object, the optic system of the eye adjusts so that the image of this object is projected on the middle part of the macula lutea, the fovea. When observing a distant point, the muscles of the ciliary body are relaxed,

the suspensory ligaments are stretched and the lens is flattened. Thus, the incoming, nearly parallel rays of light are focused on the retina. In contrast, rays of light reflected from a nearby object can be only focussed on the retina, if the convexity, that is, the refractory power of the lens is increased[2]. **The process of increasing the lens convexity is called accommodation.** When we focus on a near point, the muscles of the ciliary body contract, which leads to the relaxation of the suspensory ligaments and the lens becomes more convex because of its elasticity. Thus, looking at a near object demands active muscle work. In parallel with the *increase in convexity of the lens*, the oculomotor (striated) muscles cause *convergence* of the two eyes, the axes of the two eyeballs deviate toward each other. The third part of the accommodation process is the contraction of the (smooth) muscles of the iris, which cause the *narrowing of the pupil*. The narrower pupil allows for an increase in the depth of focus. The three above-mentioned processes are called together the **accommodation reflex or near triad**.

The nearest point, from which the incoming light rays can still be focussed onto the fovea is called the **near point of the eye**. This distance is circa 7 cm in children and it increases with the age (it is 10 cm at the age of 20 years, 25 cm at 40, 100 cm at 60 etc). To determine the near point of the eye, hold your left fist in front of yourself and focus on your upturned thumb! Approach the hand to the eye while focusing continually on the tip of your thumb until reaching the point when you cannot see it clearly any more (it is impossible to focus the image on the fovea). Measure the distance between the thumb and the eye!

The images of objects located at different distances from the eye are projected to the same retinal surface area, if their viewing angles are identical. (The viewing angle is the angle defined by the light rays coming from the two margins of the object. A double-sized object can be seen from a double distance at the same angle than a smaller object from a smaller distance.) The smallest viewing angle at which two distinct points can still be discriminated is called the *viewing angle limit*. The viewing angle limit of the healthy eye is approximately 1 angular minute (1'); this corresponds to circa 1.5 mm seen from 5 m. The *resolution capacity of the eye or the sharpness of vision (visual acuity)* is the actual viewing angle limit compared to the normal viewing angle limit of 1' expressed in percentage or decimal fraction. Thus, an eye with a viewing angle limit of 1' has a sharpness of vision value of 1 (or 100%), an eye with 2' has a sharpness of 0.5 (or 50%), etc. Visual acuity is highest at the macula lutea (circa 100%); it decreases towards the periphery of the retina.

In case of an optically perfect eye, the maximal sharpness of vision is determined by the density of photoreceptors on the retina. At the fovea centralis, the cones show a dense, honeycomb-like distribution, their average distance from each other is 2 µm. We can discriminate between two points, if their images projected onto the retina are located at least at a distance corresponding to one cone diameter (Figure. 9.9). On the peripheral surface of the retina, the rods are located with a similar density, but several rods are connected to the same ganglion cell (convergence), thus the visual acuity here is low.
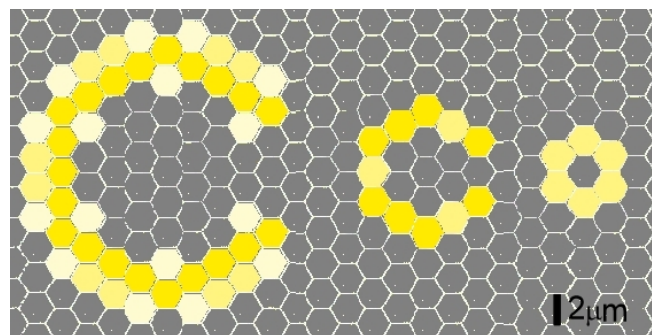
Figure 9.9. *Resolution capacity of the retina. Activation pattern on the retinal cones elicited by Landolt rings of different sizes. Inactive cones are represented by grey hexagons, while active cones are yellow. The break in the ring can be detected by the visual system only if its image has the width of at least one cone (circa 2 µm).*

During the practical, the Hungarian version of the *Snellen charts* will be used for the determination of vision acuity. The characters (letters, numbers, etc.) on these charts are arranged in lines with decreasing sizes downward. Next to each line there is a common fraction the denominator of which is indicating the distance in metres from which that row can be read by a person with normal vision. This is the distance from which the outer boundaries

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[2] The refractory index of a glass lens is a constant value; in cameras, the distance between the lens and the film is adjusted. The eyes of fish work on the same principle.

of the whole letter are seen at an angle of five minutes and the width of the lines forming the letter are seen at an angle of one minute (Figure. 9.10).
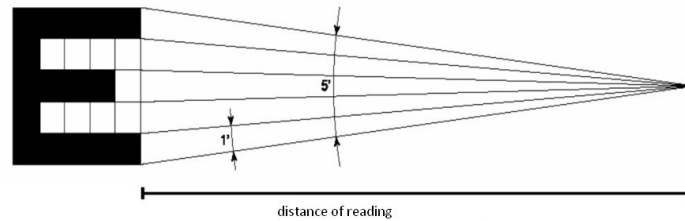
Figure 9.10. *Structure of the Snellen chart's characters. The characters are of decreasing size downwards from above. Next to each row of characters there is a common fraction, the denominator of which is indicating the distance in metres at which that row can be read by a person with normal vision. This is the distance from which the boundaries of the whole letter are seen at an angle of five minutes and the width of the lines forming the letter are seen at an angle of one minute.*

The determination of the visual acuity will be carried out from a distance of 5 m (this value is the numerator of the fractions next to the letters). The subject has to read aloud the characters downwards from above (with one eye at a time!). The observer notes the last row that the subject was able to read correctly. The visual acuity (V) is expressed by the common fraction next to this row.

9.5.4. Examination of astigmatism

Visual acuity may be decreased by the distortion or spherical flaws of the ideal spherical surface of the cornea. The most frequent problem is that the cornea has different refraction power horizontally and vertically (astigmatism). This problem may be corrected by special, cylindrical lenses that compensate the optical error of the distorted cornea. The simplest way to check the surface of the cornea is to use the ***Placido's disc*** (Figure. 9.11). There are black and white concentric circles on the disc, that reflected on the subject's cornea will become oval or distorted if the subject has astigmatism. The subject has to stand facing an appropriatelight source (i.e. a window). Look through the central aperture of Placido's disc into the subject's eye, from a distance where you can see the reflection of the rings on the cornea! Observe if you can see any distortion in the reflected circles!

Unfortunately, in everyday life, astigmatism is also called, wrongly, strabismus. ***Strabismus, squint-eye or cross-eye*** is a condition when, due to different reasons, the two eyes are not capable to focus on the same point and thus project different images into the brain. In adults, this might lead to double vision, but in children, the brain usually neglects the information coming from the weaker eye. If this condition is prolonged, the visual cortical areas corresponding to the neglected eye do not develop properly. ***Amblyopia or lazy eye*** may develop on the neglected eye, which cannot be corrected later.

Figure 9.11. *Placido's disc and its reflection on the cornea.*

9.5.5. Determination of the field of vision

The field of vision is the part of the outer world which is projected onto the surface of the retina by the eyes. Due to the anatomical form of the eye (and the retina) this would be a regular circle (or hemisphere, if we think in 3D), but different structures of the face (medially the nose, upwards the supraorbital ridge, laterally the cheekbones, i.e. the zygomatic bone) mask some parts of the theoretical field of vision.

During ophthalmological and neurological examinations, a *perimeter* is used to determine the field of vision. (Nowadays, a computerized version of the perimeter is used.) The semicircular metal arc of the perimeter is rotatable about its centre in 360°. The little disc inside this arc can be moved outwards from the centre until 90° (Figure. 9.12). The subject should place his chin on the chin-rest and the height of the seat should be adjusted until the eye to be examined is on the same level as the centre of the device. The other eye should be covered.

Put the appropriate test paper corresponding to the examined eye into the holder! Move the small disc slowly from the periphery towards the centre of the field of vision, while the subject focuses on the centre! The subject should indicate when he glimpses the disc. This point should be registered on the test paper (if the paper holder is lifted, it is pierced by a metallic needle at the correct location). The test paper is scaled in every direction up to 90°. The field of vision is 100 % in a given direction, if the disc is detected even if moved farther then 90°. Repeat the measure in every spatial direction with steps of 30°! Connect the registered points! Compare the field of vision of the subject with the average field of vision plotted on the test paper! Try to explain the differences, if any!
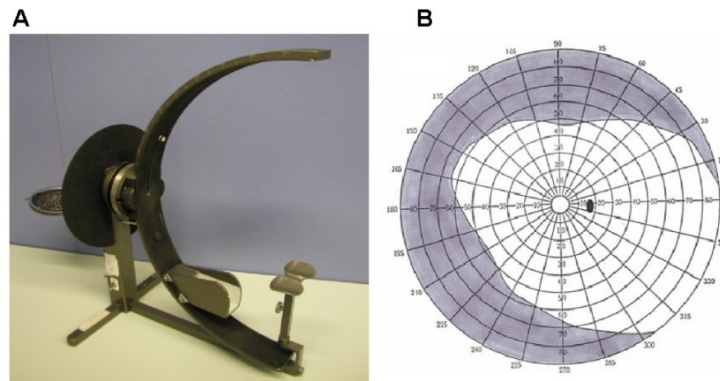
Figure 9.12. *The perimeter used at the practical. The semicircular arc of the perimeter is rotatable about its centre in 360°. The little disc inside this arc can be moved outwards from the centre until 90° (A). A test paper designed for the right eye. The field of vision is 100 % in a given direction, if the disc is still detected even if it moved outwards by 90°.*

Determine the location of the blind spot with the perimeter! In our field of vision, the blind spot is located in temporal direction from the macula, by approximately 1.5^o below the horizontal direction, at about 12-17 ^{o} from the center (it is marked on the test paper). Determine the field of vision also with coloured test discs. Examine whether there is any difference in size between the black-and-white and coloured (green, blue or red) fields of vision! What is in the background of the differences?

9.5.6. Characterization of the blind spot

On the computer, you can find a document with a simple test demonstrating the presence of the blind spot. When you open the document, you will see two characters. Measure the distance between the two characters on the monitor (d) using a ruler! Focus with one eye on the nasal character (closer to our nose)! Approach to the monitor until the temporal character (farther from the nose) disappears from the field of vision! Measure now the distance between the eye and the monitor (x_1)! Approach further until the distal character becomes visible again! Draw back a little bit until the character disappears again. Measure the distance between the eye and the monitor (x_2) once more! Calculate the distance between the blind spot and the macula (d'), and the size of the blind spot (Δd), using the Figure 9.13., and the simplified formulae below:

$$d'_1 = 17 \, d/x_1 \quad d'_2 = 17 \, d/x_2 \quad \Delta d = d'_1 - d'_2$$
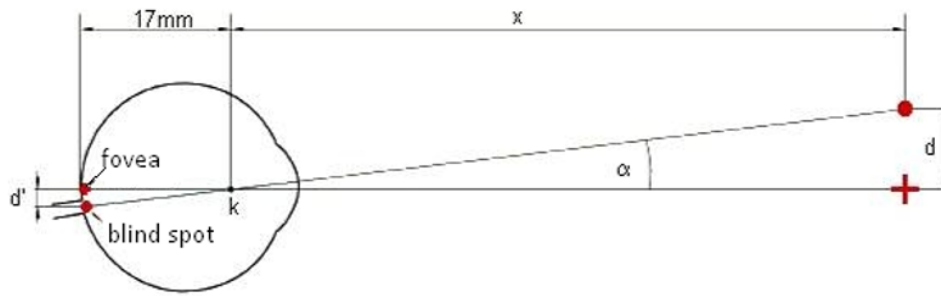
Figure 9.13. *Calculating the distance between the blind spot and the macula (d'). d: distance between the two characters of the document; x: distance between the eye and the monitor measured at the disappearance of the distal character. The eye's optical system is much more complex than in this model, but for simple calculations, it can be replaced by a so-called "reduced eye". The reduced eye is a homogenous body having one refractory surface, where the centre of the spherical refractory surface (k) is located at 17 mm from the macula.*

9.5.7. Investigating colour vision

In the retina of colour-blind people, receptors detecting one of the three basic colours (red, blue, green) are of decreased sensitivity or missing, thus they cannot detect or distinguish certain colours, usually the complementary pairs, like red-green, blue-yellow. According to statistical data, 8 % of men and 0.5 % of women are colour-blind. Colour perception is usually examined with Stilling-Ishihara plates (Figure. 9.14). These plates contain a character (letter or number) or a shape in a colour different from that of the background. Both the figure and the background consist of dots to eliminate the contours of the figure. The average size and lightness of the dots is equal in case of the figure and the background, so the perception of light contrast or pattern cannot help to recognize the figure. Thus, the figure is invisible, or difficult to see, to those who cannot distinguish the colour of the figure from that of the background. There are three basic types of colour blindness. From the retina of dichromatics, one cone type is missing. They cannot distinguish certain colours. In the eye of the monochromatics, there is only one cone type; they do not detect colours, only differences in luminosity. In the eye of the rod-monochromatics, there are no cones at all. They cannot distinguish colours and they have to wear dark glasses in daylight (without this, their rods would saturate and they would not see anything). The most common colour perception disorder is red-green colour blindness (dichromacy). The gene coding for the pigment protein of red and green cones is located on the X chromosome; that is why this condition is more frequent among men. (The gene of the blue cone pigment is on chromosome 7, while the gene of rhodopsin is on chromosome 3.)
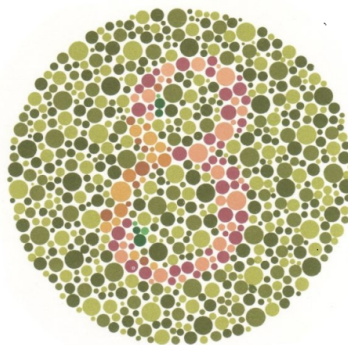
Figure 9.14. *Stilling-Ishihara plates contain characters (letters or numbers) in a colour different from that of the background. Both the figure and the background consist of dots to eliminate the contours of the figure. The average size and lightness of the dots is equal in case of the figure and the background, so the perception of light contrast or pattern cannot help to recognize the figure. Thus, the figure is invisible, or difficult to see, to those who cannot distinguish the colour of the figure from that of the background.*

The experiment has to be carried out in diffuse daylight. First, the control plate is presented, which can be recognized even by completely colour-blind people, because either the characters are made up by dots that differ in contrast from the background, or the contours of characters are easily discernible. Then, the test plates are shown one by one; these are recognized by people with normal colour vision within 3-5 sec.

The **laboratory report** has to be prepared using the form in the Appendix. Attach to the report the drawing made from the eye model! Mark the blind spot and the macula on the drawing. Are the blood vessel shadows seen in the Purkinje experiment similar to the blood vessels seen in the eye model? Determine if the subject's eye has a near point corresponding to his or her age. Analyse the results of the experiment about visual acuity! Draw an eye model in the report and mark into which point the optical system of the subject's eye focusses the image of an object. Draw with different colours the condition without optical correction and, if correction is needed, the condition with appropriate correction. Attach to the report the perimeter chart showing the subject's field of vision! Compare it with the average field of vision plotted on the chart. Try to explain the eventual differences. Compare the sizes of black-and-white and colour fields of vision. Explain the differences. Compare the data concerning the blind spot (size, distance from the macula) obtained with the perimeter and with the computer program. Do the data correspond to the expected values? Did the subject recognize the figures on the Stilling-Ishihara plates? If not, which colours were problematic?

9.6. Electrooculography (EOG) with the Biopac recording system

One primary motor mechanism of the visual system is to keep the observed object within our field of vision. This process should work even when our head or the object is moving. The visual system positions the eye with the help of the extraocular muscles (four rectus and two oblique muscles), so that the image of the observed object is projected onto the retinal area of greatest acuity, the fovea centralis. The movement of the eyes can be voluntary, when we lock/fixate our gaze on an interesting object. Voluntary eye movements usually occur when choosing the object, then, involuntary/spontaneous eye movements keep this object within the visual field.

If we fixate an immobile object within our field of vision, the eyeball carries out small involuntary movements. This motion may be: (i) tremor with a frequency of 30-80 Hz, (ii) slow drifting movement, when the image of the object drifts across the fovea, (iii) quick flicking reflex movement, which brings the image of the object back to the fovea. The drifting and flicking movements are usually of opposite direction.

When we follow a moving object with our gaze, it is the speed of the object which determines the type of eye movement. If the object is moving slowly, the eye uses **slow tracking movement** to keep its image continually on the fovea. If the object is moving quickly, the eye fixates its image again and again. Between the fixation points, the eye carries out rapid involuntary movements, so-called **saccades**. Saccadic movement is the most rapid movement of the human body; its speed may reach 900°/s. This type of eye movement occurs not only when tracking a quickly moving object but also during reading or while looking at a picture. After fixing a word or a part of the picture, our eye jumps quickly to the next one via saccadic movement.

Eye movements may be studied using the electrooculography (EOG) method. The electrophysiological basis of the EOG technique is that our eyeball is an electrical dipole: the positive pole is located at the surface of the cornea, while the negative pole is behind the retina. (That is why, besides examining eye movements, electrooculography is used to examine alterations of the pigment cells also.) The potential difference between the two above-mentioned points is of 0.4-1.0 mV. This corneo-retinal potential is created partly by the Donnan equilibrium between the intraocular liquid and the blood, and partly by the effect of light on the receptor cells (rods and cones). This potential difference creates an electric field around the eyeball; the orientation of this field changes during eye movements. The potential difference can be registered with electrodes placed around the eye and examined after appropriate amplification. Horizontal eye movements can be recorded with electrodes placed on the temples, while vertical movements with electrodes placed below and above the eyes. Reference electrodes are usually placed on the middle of the front or on the earlobes. This method allows the recording of eye movements up to $\pm70^o$ (0° corresponds to the direction straight ahead, while $\pm90^o$ to the maximal horizontal or vertical deviation).

Experimental methods

Attach the adhesive electrodes on the front and cheeks of the subject according to Figure 9.15, then connect them to the vertical and horizontal leads (take care of the colour code)!

Make sure the Biopac MP30/35/36 unit is turned off. Plug the leads of the EOG electrodes into the amplifier unit: horizontal lead into channel 1 (CH1), vertical lead into channel 2 (CH2), then turn the amplifier on. Start the Biopac Student Lab program and choose Lesson 10 (Lesson 10: EOG I).

To start the calibration procedure, click **Calibration** and follow the instructions on the pop-up windows! The subject should fix his/her gaze at the centre of the screen, then follow the blue dot with his/her eyes, without moving the head. If calibration is unsuccessful, it may be repeated by clicking **Redo calibration**. If calibration is successful, proceed to data recording.
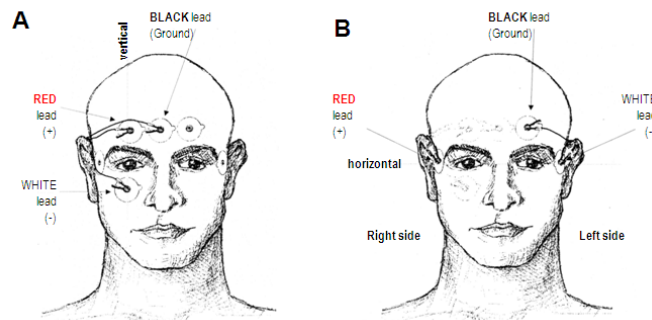
Figure 9.15. *Positioning and attachment of vertical (A) and horizontal (B) leads.*

Recording tasks

9.6.1 Examination of slow tracking eye movements

First tracking eye movements are recorded. Start recording by clicking **Record**. The observer should keep his/her finger at the centre of the subject's field of vision for approximately 10 seconds. The subject should fixate onto the finger tip. Then the observer should slowly move his/her finger to the right, back, upwards, back to the centre, etc. (The subject should not move his/her head!) Before each change of direction, the observer should hold his/her finger motionless for 2 seconds. At each change of direction, a marker should be inserted into the recording (F9 key). If the recording is finished, stop it by clicking **Suspend**!

9.6.2 Examination of saccadic eye movements during reading

Continue recording by clicking **resume**. The subject should read the attached sample texts. Analyze the effect of font size and type on reading! The subject should also read texts with the same typography but of different levels of difficulty to understand: fairy tale, biological technical text in Hungarian and in English. How do the differences in interpretation difficulty influence reading and eye movements? Correct recording should be stopped by clicking **Stop**.

9.6.3 Eye tracking - examination of fixation patterns

During this part of the recording, the computer plots the points fixated by the subject on the screen. After appropriate calibration, this method allows to plot which motives, parts of a picture are observed more thoroughly than other parts. Recording can be started after finishing the two previous steps by clicking **DotPlot**. The subject should fixate onto the centre of the screen and move his/her head until the green point indicating the target of fixation is positioned in the centre of the screen. The subject should keep this head position and observe the picture which the observer holds in front of the screen. The recording can be stopped by clicking **Stop**. The program shows the points fixated by the subject during the last 30 seconds. If the setup was correct, the fixated points appear more densely at the image motives "interesting" for the viewer (angles, edges, contrasts).

Preparing the laboratory report

1. Identify the different types of eye movements on the recording! Zoom in and attach the corresponding segments! Analyze the phenomena seen on the recording.

2. In case of each recording representing the reading of different texts (different difficulty; font size; font type), measure the duration of the first 8 saccades! This can be done by zooming onto the appropriate segment and set up in the measurement box the following parameter: channel 41 – delta T. Measure the duration of the saccades using the I-beam tool of the software. Calculate the time taken up by all the examined saccades and measure the time necessary for the reading of the whole line. Calculate the percentage of time represented by saccades.

3. After opening the file, zoom into the recording! Attach the recording and the image showing the fixation points to the lab report. Describe your experiences! Which physiological phenomenon characteristic for the retina may be demonstrated using this method? What is the significance of this function?

Chapter 10. Examination of biolectrical signals accompanying brain function (EEG)

10.1. Introduction

One of the important functions of the *nervous system* is to organize the regulation of the different physiological functions on the level of the *organs*, *organs systems* as well as on the level of the *whole organism*. Besides the regulation, another equally important function is the *analysis of the stimuli* arriving both from the outside world and the body. Associative processes regarding time and space context of the stimuli lead to memory formation and learning, both are very important function of the central nervous system as well as generation of emotions and motivations. These higher order functions are associated with the activity of the cortex. With the method of the *electroencephalography*, it is possible to measure bioelectrical potential changes accompanying brain functions. The tool for this examination is the *electroencephalograph* which records wave series called as *electroencephalogram* (EEG). In the first time, Richard Caton recorded EEG in rabbits in 1875 followed by Hans Berger who recorded first time electrical activity from human scalp. In 1929, Berger defined the major frequency bands (α, β, θ and δ bands) by which the pattern of brain activity is conventionally characterized. Gamma (γ) activity or gamma oscillation was described later. It contains characteristic sine waves and closely associated with cognitive functions. This rhythm usually can not be recorded from the scalp; it requires the usage of electrodes placed directly to the cortical tissue. It can be peformed in animal models or in specific cases in humans.

Field potential recordings provide an important tool for qualitative and quantitative analysis of neural population activity. Transmembrane currents of neurons with synchronized activity flow through the extracellular space and these currents can be recorded as field potentials by appropriate electrodes. One of the most extensively studied field potentials is the EEG which examination serves also diagnostic purposes and it had own evolution from the '30s parallelly with the development of electrical engineering.

Waves representing potential changes have different frequencies. These waves can be recorded with electrodes placed to the scalp or attached directly to the cortical surface. The amplitude of these macropotential waves, when recorded from the scalp, is in the 10 μ V magnitudes. During synaptic activity, positive (inward) current flows through the dendritic membranes into the cell generating an active „sink". Current flows into the opposite (outward) direction through the membrane of the soma (so called passive „source"). When depolarization spreads to the soma, position of the sink and source is swapped. The sink and source form a dipole, and the circuit will be closed through the extracellular space. The main sources of the EEG signal are these dipoles generated by slow synaptic potentials (EPSPs and IPSPs), but non-synaptic currents such as burst-evoked afterhyperpolarizations also contribute. Action potentials usually do not play a role in generation of the EEG as they have a short duration (<2 ms) and only a small membrane surface is involved in their generation. It is important to note that the extracellular space of the neural tissue is not conductive to high-frequency electrical waves which hamper the spatial summation of the high-frequency signals. Neuronal geometry also plays a role in the EEG generation. Neurons with concentrically situated dendrites can not produce macropotential changes as dipoles with different directions will quench each other. Field potentials can be easily recorded from structures containing elongated cells. The cortex and the hippocampus are ideal structures where large pyramidal cells meet the above mentioned morphological criteria. Potentials of individual cells have very small amplitude. If slow membrane potential fluctuations in neighboring cells are ordered in time, high-amplitude and low-frequency waves appear. This phenomenon is called *synchronization*. If electrical activation of the neurons is not coordinated, signals from individual neurons quench each other resulting in low-amplitude and high-frequency waves, i.e. *desynchronization*. Appropriate amplification is also an important factor for recording EEG signals.

Actual EEG patterns depend on the actual sleep-wake stage (Figure 13.1). Several so-called *ascending activating systems* were identified by extensive research. These systems (serotonergic, noradrenergic, histaminergic, GABAergic and cholinergic from the basal forebrain etc.) originate from subcortical regions and influence the electrical activity of the cortical neurons by modifying their membrane properties. Thus, activation level and

activity pattern of the cortex changes periodically during the cyclic alternations of the sleep-wake stages, which can be followed by EEG recordings.

During wakefulness, beta- and gamma waves can be seen. However, alpha activity can also be developed if external stimuli are minimised (eyes closed, quiet environment). During slow wave sleep, low frequency waves are characteristic and *synchronization* processes can be studied. In the case of sensory stimulation or awakening, beta activity develops, i.e. *desyncronization* occurs. Desynchronized patterns are also characteristic in paradoxical (REM) sleep. However, specific waves appear during this stage (so-called sawtooth waves). These waves are missing during wakefulness.
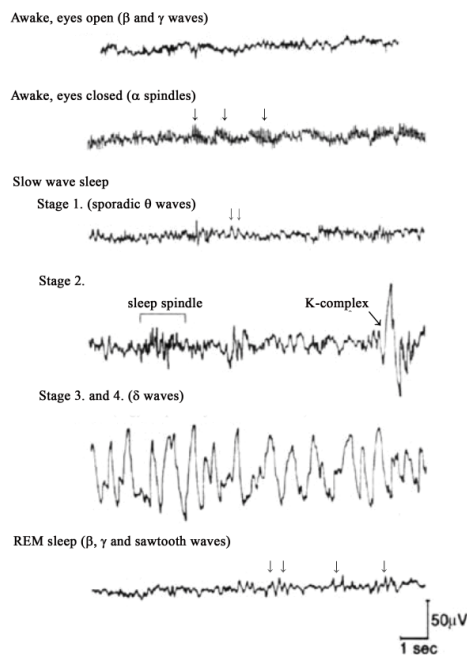
Figure 10.1. *EEG patters characteristic to different sleep-wake stages in a healthy, adult person*

EEG can be routinely measured by macroelectrodes which have large surface and low electrical impedance. In the course of the practical lesson, human EEG during wakefulness will be recorded and analyzed.

10.2. Human electroencephalography

EEG method has high diagnostic importance in clinical practice, especially in neurology. EEG measures have several advantages as they provide immediate information about the actual condition of the cortex by a non-invasive way. In routine examinations, brain activity can be checked by using only 6-8 electrodes. However, in more sophisticated and accurate measures, usage of 32 or even 64 electrodes is a requirement. Silver macroelectrodes are used routinely.

Placement of the electrodes on the scalp is the first step in human EEG recordings. Before the fixation of the electrode, unfolding the hair on the place of the recordings and removal the grease from the skin surface with alcohol is needed. Depending on the electrode type, conductive gel or paste is applied to ensure good electrical contacts. Electrodes can be fixed by a rubber cap or even with a piece of gauze which is glued to the skull by a special glue (Mastisol). In the practical lessons, usage of an elastic gauze bandage is a very useful alternative. It is placed on the subject's head to fasten the electrodes and their leads. Good electrical contact can be achieved in this way.

In the clinical use, EEG is recorded from conventionally defined locations (see Figure 10.2). This is the so-called *international 10-20 system,* which defines four anatomical reference points on the skull. These are the nasion (depressed area just above the bridge of the nose), the inion (the lowest point of the skull from the back of the head, indicated by a prominent bump at the level of the *foramen ovale*) and the two praeauricular point (place of the mandibular joint on the right- and on the left side, respectively). Distances are measured between the above mentioned reference points and electrodes are placed on the 10 and 20 % dividing points of these distances. In the 10-

20 system, locations are marked with capitals (F – frontal, P- parietal, C – central, T – temporal, O – occipital). Numbers followed by the capitals mark the hemisphere (even numbers - right hemisphere; odd numbers - left hemisphere), while lowercase "z" marks central position. Reference/ground electrode is provided by a clip fixed on the earlobe.

Taken into consideration the conditions present on the practical lessons, only EEG patterns characteristic to wakefulness can be recorded. Thus, only alpha- and beta activity can be demonstrated. It is possible to manipulate alpha activity by some simple ways and artefacts can be also demonstrated.
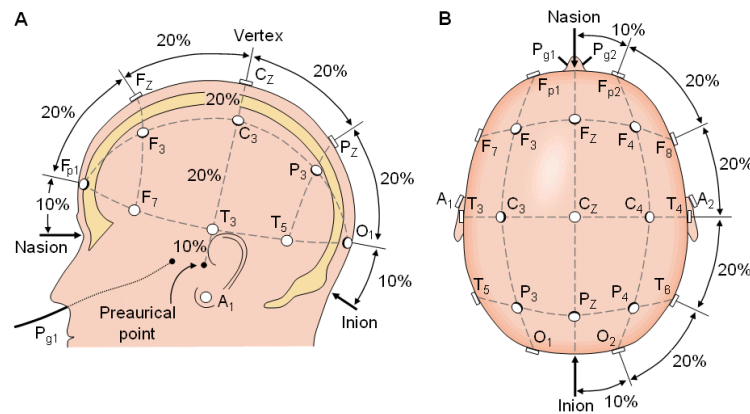
Figure 10.2. *The international 10-20 system seen from the left side (A) and from above the head (B). A - ear lobe, C - central, Pg - nasopharyngeal, P - parietal, F - frontal, Fp - frontopolar, O - occipital electrodes.*

If the subject closes her/his eyes and lie in complete mental ("think about nothing") and physical rest, alpha activity appears in the EEG characterized by a frequency of 7-13 Hz and an amplitude of about 50 µ V. Alpha rhythm can be preferentially recorded from occipital and posterior parietal areas. Audience should be quiet during the recording.

When a continuous alpha activity is present in the EEG, and the subject is asked to *open her/his eyes*, alpha activity disappears and beta rhythm appears with a frequency higher than 13 Hz and with amplitude lower than 50 µ V. Thus, desynchronization occurs. Desynchronization of the continuous alpha activity can be evoked by several ways: by sound stimuli (for example: clap), by tactile stimulation of the skin (for example: touching the hand of the subject) or by forced mental activity (for example, to ask the subject to solve a simple arithmetic task without saying the result aloud).

Voluntary hyperventilation has a strong influence on alpha activity. To test this phenomena, the subject should hyperventilate for half a minute (breathe with maximal frequency and amplitude). Hyperventilation can provoke seizure in epileptic subjects. Thus, it is important to skip this test if the subject has previous record of seizures!

During EEG measurements, it is very important to minimize the amount of artefacts or at least to exactly identify the appearing ones. Artefacts can be very similar to normal EEG waves. For example, eye movements can result waves mimicking delta waves or K-complexes characteristic to slow wave sleep, even if the subject is awake.

The most frequent artefacts originate from eye movements appearing on the EEG electrodes close to the eyeballs. To examine this artefact, subject is asked to move her/his eyeballs slowly to the left then to the right with closed eyelids. Function of skeletal muscles can also cause characteristic artefacts. It can be demonstrated by asking the subject to tense her/his mandibular muscles. Effect of vocalization can be introduced by asking the subject to pronounce her/his name.
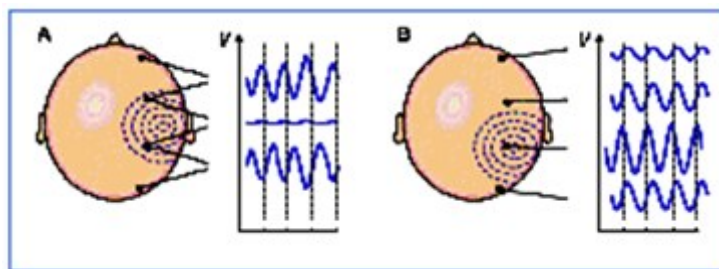
Figure 10.3. *Shape and amplitude of the EEG waves depends on the recording location as well as on the way of the measurement. (A) Bipolar and (B) unipolar measurements. In the latter case, potential of the active electrodes is measured relative to a common reference electrode. Monopolar measurements are mainly used for research purposes. Popular grounding points: nose, ear, connected mastoid processes, forehead. Noise reduction is the aim of the grounding.*

During the quantitative analysis of the EEG signal, recordings are divided into consecutive epochs with pre-defined duration (usually 4 – 20 seconds) then Fast Fourier Transformation (FFT) algorithm is performed. FFT is a mathematical algorithm, which is used frequently in physics. The principle of FFT lies in the hypothesis that EEG waves appearing in the pre-defined epoch have different frequency, amplitude and phase values and can be described as a sum of sine and cosine waves. Result of the FFT shows quantitatively the amplitude values of waves belonging to the different frequencies as well as their phase information. From the transformed curves power spectra can be calculated that show the intensity or power of the different frequency components of the analyzed EEG epoch. The original EEG signal can be reconstructed from the data provided by the Fast Fourier Transformation, but not from the power spectra, as the phase information is lost during its calculation.

10.3. Single-channel human EEG recording using Biopac Student Lab system

Preparations for the measurement

Attach one electrode to the earlobe of the subject for grounding purpose. Make sure that the electrode has appropriate contact with the skin. Electrode should contain appropriate amount of paste for solid electrical contact. Grounding electrode can be placed on the neck instead of the earlobe. According to Figure 10.4A, place two additional electrodes on the scalp for recording EEG. Place supportive rubber cap or elastic gauze bandage to press electrodes against the scalp. Plug electrode lead set (SS2L) according to Figure 10.4B. Pay attention to the color code!
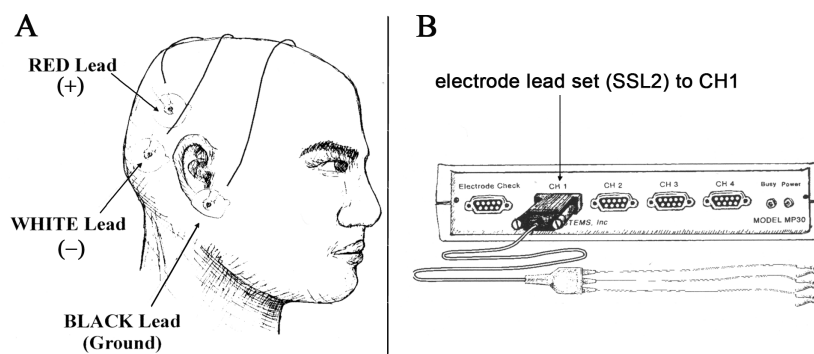
Figure 10.4. *Electrode positions on the head and their color-coded connections (A), electrode lead set connection with the Biopac hardware (B)*

Make sure the Biopac MP30/35/36 unit is turned off. If not, turn it off. Plug electrode lead set to channel one (CH1). Turn the MP30/35/36 unit on.

During the recording, subject should be in supine position. Comfortable position is a must as subject should be motionless throughout the recording session.

Recording protocol:

1. *Demonstration of the EEG components belonging to different frequencies. Effect of visual information on the parieto-occipital EEG*

Start Biopac Student Lab software and choose Lesson 3 (EEG I).

Start the calibration process by pressing the **Calibrate** button. Follow the instructions on the pop-up windows. During the calibration, 8 seconds of EEG signal is recorded. In case of a successful calibration, there should be a low-amplitude signal (around the zero value). If the recorded signal is different, redo calibration by clicking the **Redo Calibration** button. If the calibration succeeded, recording can be started.

Tasks:

- At the beginning of the recording, subject should close her/his eyes. Press **Record** button to start recording.

- After 20 seconds, subject should open her/his eyes. The observer should place a marker in the file by pressing the **F9/F4** button.

- After another 20 seconds long period, subject should close her/his eyes again. The observer should place a marker in the file by pressing the **F9/F5** button.

- After 20 seconds of recording, terminate the measurement by pressing the **Stop** button.

If the recording was performed well, recording should resemble the picture seen on Figure 10.5. The software shows EEG components belonging to different frequency bands if buttons representing these bands (alpha-theta) are clicked (see Figure 10.5.B).
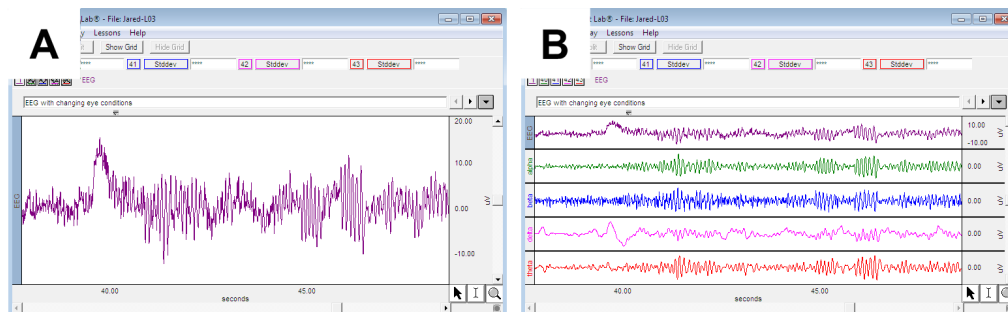
Figure 10.5. *The recorded EEG signal (A) and the signal decomposed into its frequency components (alpha-theta) (B)*

The recording was succesful if alpha activity decreased after eye opening. If it is not the case, then some error might have occurred, and the recording should be repeated by pressing the **Redo** button. By pressing the **Done** button, recording can be terminated.

2. Changes of the EEG alpha rhythm

Start Biopac Student Lab software and choose Lesson 4 (EEG II).

Start the calibration process by pressing the **Calibrate** button. Follow the instructions on the pop-up windows. During the calibration, 8 seconds of EEG signal is recorded. In case of a successful calibration, there should be a low-amplitude signal (around the zero value). If the recorded signal is different, redo calibration by clicking the **Redo Calibration** button. If the calibration succeeded, recording can be started.

During the recording, changes of alpha rhythm will be examined in different conditions:

1. relaxed state with eyes **closed**

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2. performing **mental math** with eyes **closed**

3. recovering from **hyperventilation** with eyes **closed**

4**. relaxed** state with eyes **open**

The software shows the recorded EEG, the alpha frequency band of the EEG signal and alpha power (as alpha-rms - root mean square). Alpha-rms is also visualized by a column graph.

Tasks:

- Start recording by pressing the **Record** button. Record a 15-20 seconds long segment when the subject is relaxed with eyes closed. Press the **Suspend** button to stop the recording.

- The observer should prepare the math task. The task should be neither too easy nor too difficult. It should contain operation with fractions.

- Continue recording by pressing the **Resume** button. The observer should give the subject the math task. Subject sould perform mental arithmetic but the result should not be said aloud.

- After 20-30 seonds, recording should be stopped (press the **Suspend** button). Result of the mental arithmetic should be discussed.

- Suject should hyperventilate for a minute with eyes closed. Immediately after the end of the hyperventilation, recording sould continue again (**Resume**). Subject should be in a relaxed, supine position with eyes closed.

- After 10 seconds, recording should be stopped (**Suspend)**.

- Subject should open her/his eyes and should be in a relaxed, motionless position for 2-3 minutes.

- Start recording again by pressing the **Resume** button then press **Suspend** after 15 seconds of recording.

- Press **Done** to terminate recording.

Data analysis: After opening the file recorded with Lesson04 (EEG II), the following signals can be seen on the screen: Channel 1 – recorded (raw) EEG signal; Channel 40 – alpha frequency band (after digital filtering); Channel 41. – alpha rms. The alpha rms (root mean square) value is the square root of the mean values (calculated in 0.25 s long time windows). It is a good indicator of the actual amount of alpha waves.

1. Determination of the amplitude and frequency of alpha waves

During the analysis, channel 1 (raw EEG) data is examined. Zoom in on a small segment then set the amplitude for optimal viewing by the **Display/Autoscale waveforms** function. Set the following measurement windows: first window - channel 1 (raw EEG) – p-p (peak-to-peak amplitude); second window – channel 1 (raw EEG) – delta T. Identify consecutive alpha waves in the recording with sinusoid shape then select an alpha wave by the **I-Beam** cursor from the onset of wave to the peak of the wave. This selection marks the amplitude of the wave. Note the amplitude value seen in the first measurement window (channel 1 –p-p). Following this, select the same wave from beginning to end. In the second measurement window (channel 1 – delta T), period time of the wave appears. During the calculation of the delta T parameter, selection order matters as delta T value is positive when the selection performed from beginning to the end while negative if the direction of the selection is the opposite. Repeat the above mentioned process in case of another 7 different alpha waves then calculate the frequency of the waves using the period time values. Calculate the average of the amplitude, period time and frequency values of the analyzed waves.

2. Determination of the amplitude and frequency of beta waves

During the analysis, channel 1 (raw EEG) data is examined. Steps of the analysis are similar to ones described in the previous section. Calculate the average of the amplitude, period time and frequency values of the analyzed waves and indicate the averages in the table.

3. Effect of different treatments on the amount of alpha waves

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During the analysis, Channel 41 (alpha rms) data is examined. Set the following measurement window: channel 41 (alpha rms) – mean. Zoom in on the recording then set the amplitude for optimal viewing by the **Display/Auto-scale waveforms** function. Based on the signal in channel 1 (raw EEG), select 2-second long segments from the recording of the consecutive tasks, then note the alpha rms values displayed in the measurement window.

During the selection of the analyzed segments, the first, control segment should contain a significant amount of alpha waves as it can be seen in channel 1 (raw EEG). During the selection of the second segment (mental arithmetic), select the segment recorded immediately after the subject got the math task. It can be hypothesized that the subject concentrated most on the task during this period. Selected segments must be free of artefacts. Alpha rms value of the first segment (relaxed state with eyes closed) serves as control. Compare alpha rms values of the second, third and fourth segments (hyperventilation, opened eyes) to the control one and indicate them in a table.

10.4. Examination of previously recorded and printed EEG curves

Printed EEG recordings of subjects with different age and health status are available on the practical lesson. Age-dependent variations as well as pathological patterns can be studied using these recordings.

Traditional analysis of the EEG recordings is performed visually. Type of activity (alpha, beta etc.) can be identified easily by visual observation. Presence of characteristic waves or patterns (K-complexes, spikes, sleep spindles etc.) can also be easily recognized. Frequency analyis of alpha oscillation can be performed manually. However, this method is very slow, difficult and inaccurate. This task is done by computer nowadays.

Age-dependent variations of the EEG

Brain electrical activity changes in parallel with development. EEG patterns characteristic for childhood markedly differ from those in adults. EEG signal is very variable even in adults, but this feature is more pronounced in children.

Below 1 year age, slow waves characteristic to sleep in adults also appear during waking. At 3 months, 3-4 Hz rhythm is dominant in the posterior regions and this rhythm becomes faster (6-7 Hz) when the infant reaches the age of 1 year. If the infant is sleepy or is napping, the dominant frequency decreases by 1-2 Hz. Transition to sleep is slow and can last for minutes. Decrease in the number of artefacts shows the process. Below the age of 6 months, characteristic, high-amplitude theta rhythm appears during the transition. Sleep spindles appear at about the age of 2 months in the sleeping infant. Spindles have a frequency of 12-15 Hz. These are asymmetric at the sleep onset, but become more symmetric with sleep progression. K-complexes and sharp waves appear in central cortical regions from the age of 5 months. Sleep spindles are superimposed on the background of the 0.75-3 Hz delta rhythm.

As the child grows, EEG activity during waking becomes faster. In a healthy 8-year old child, waking rhythms have an at least 8 Hz frequency. Between 6 and 12 years of age, so-called *lambda waves* are present in the waking EEG in the occipital cortex, appearing when a complex visual stimulus is presented. These waves have duration of 200-300 ms and disappear with closed eyes. *Mu, or μ-waves* are also characteristic at this age. These waves can be recorded from the central areas, have a comb-like shape and a dominant 7-11 Hz frequency. Movement (or even thinking about movement) inhibits the appearance of μ waves.

During naps, alpha activity is not pronounced between 6-12 years of age; theta and delta waves can be seen instead of alpha waves. As the age progresses, appearance of the so-called *hypnagogic hypersynchrony* decreases. This pattern may be present from 3 months of age to 13 years of age. During falling asleep, high-amplitude and very rhythmic series of waves with a 3-5 Hz frequency can be recorded prefrontally and centrally. These series last for 0.5-1.5 seconds. Between 6 to 12 years of age, the so-called *POSTs* (*positive occipital sharp transients* of *sleep)* appear in the first stage of the slow wave sleep. These waves are similar to the lambda waves seen during waking. POSTs are only present in a fraction of the children (and adults, later). *Sleep spindles* and so-called *sharp vertex transients* are present in the second stage of slow wave sleep. These transients are similar to K-complexes, but have shorter duration, steeper slope and are spatially more localized (central regions; *vertex* means top of the head). The so called "*14 & 6 positive spikes"* may also be present during sleep. These waves belong to the group of *epileptiform variants on the normal EEG*. This group involves waves or wave complexes that appear frequently in children and are regarded as indicators of epilepsy or other neurological diseases in the past. However, later studies

proved that these waves have no relation to diseases. Thus, they are regarded now as non-pathologic variants of the normal EEG patterns. Among others, *phantom spike-waves, psychomotor variants* and *midline theta rhythm* belong to this group. These are found to be present in the normal, healthy population with an incidence of 10-15 %.

During adolescence (13-19 years), EEG patterns become more similar to the adult ones. Alpha activity becomes much more regular. Theta and delta waves gradually disappear from the waking EEG together with other waves belonging to these frequency bands (for example, sharp waves). EEG pattern characteristic to the adult person evolves completely by the end of adolescence.

Pathological EEG patterns

Pathological EEG patterns can be present independently from age and are classified to the following groups: *epileptiform activity, background slowing, amplitude abnormalities, specific patterns and patterns resembling to normal activity but different from those in some aspects*. Epileptiform activity is the easiest one to recognize. In the diagnosis of the epilepsy, EEG measurement remains the fundamental method even nowadays.

Besides stroke, epilepsy is the second most frequent neurological disorder. In fact, epilepsy is a common and diverse set of chronic neurological disorders characterized by seizures. It can be a separate disorder or a symptom of another disease (*symptomatic epilepsy*). In case of epilepsy, one or more neuronal group becomes hypersynchronized and hyperactive and a so-called *epileptic focus* evolves. Pathological activity is initialized from the focus. Abnormal EEG waves appear in parallel with the pathological neuronal activity. Among these abnormal waves, so-called *spike waves or spikes* are the most characteristic. In a part of the cases, spikes are followed by a slow wave (*spike and slow wave*). In case of *focal (partial) epilepsy*, disordered activity is only unilateral and even in the involved hemisphere; the activity is restricted to a given area. Thus, it appears only on a subset of electrodes. In *generalized (bilateral) epilepsy*, pathological activity evolves at least at minimal level in both hemispheres, and can be recorded from the whole cortex.

Epileptic EEG can be divided into two parts: *ictal part* means EEG recorded during the seizures, while *interical part* contains EEG between the seizures („ictus" means seizure). Interictal EEG can be normal. Epilepsy is a very versatile disorder and many epilepsy types were defined in the medical practice. Three types will be introduced here in details.

Complex partial seizure is the most frequent seizure type in adulthood. 60-70 % of the cases fall into this category. This type was regarded as *temporal lobe epilepsy* in the past. Low seizure threshold of the temporal lobe and the medial limbic structures (hippocampus) is thought to play an important role in the development of this disorder. As temporal lobe has a variety of functions, very diverse symptoms may appear. Among these, are visceral symptoms (for example, diffuse discomfort feeling spreading from the stomach upward), perceptual illusions, cognitive dysfunctions (incoherent speech, speech arrest, hallucinations etc.), affective problems (anger or anxiety during seizure) and motor symptoms. The latter is seen usually as some kind of automatic movements (chewing, gulping, recurrent manipulation of an object, repetition of a word or a sentence etc). During the seizure, disturbance of consciousness develops, which varies in severity. In case of a milder disorder, partial connections with the environment may persist. Duration of seizures varies between 30 seconds and 2 minutes. In the EEG, sharp waves can be seen in the frontotemporal regions at a 8-8,5 Hz frequency. In milder cases, spikes or spike and slow wave complexes appear only sporadically.

Absence or petit mal type epilepsy belongs to the group of generalized epilepsies. Ictal activity consists of bilateral spike and slow wave complexes with 2-4 Hz frequency. Seizures are short (30 seconds maximum) with sudden onset and offset. Interictal activity is normal and symmetric between the hemisperes. However, spikes and spike and slow wave complexes may appear sporadically even in this state. Absence seizures are present in many children but in the majority of the cases (approximately 70 %) seizures vanish by 18 years of age.

The most well known seizure type is the *grand mal* seizure and it also has the most frightening symptomes. Grand mal seizure consists of a *tonic phase,* that is short in duration, and is characterized by continuously streched muscles (respiration may be blocked) and a *clonic phase* characterized by continuous muscle contractions and relaxations. Seizure begins with spikes or with a generalized and large increase in EEG amplitude. During the tonic phase, diffuse and large-amplitude muscle contraction artefacts may be present in the EEG, and the frequency of spikes continuously increases to 8-10 Hz. In the clonic phase, slow waves appear between the spikes hence frequency decreases from the initial 4-5 Hz to 1 Hz. Seizure duration is usually 1-2 minutes. Interictal EEG may be normal.

However, in many cases, *polyspike and waves complexes* appear, when one slow wave is asscociated more than one spikes.

Lab record

EEG recordings made previously using the international 10-20 system should be analyzed according to the table attached to the recordings. Recordings contain a calibration bar and the age of the subject. One recording from a healthy subject and another from a subject with some neurological problem should be analyzed.

Example for the identification of the recording loci:

1. channel: Fp1-O1 means *frontopolar1-occipital1*

For the calculation of the characteristic waveforms' frequency, calibration bar should be used. First, mark the analyzed segment on the recording (for example, a representative segment containing an alpha-spindle) then count the waves present in the segment. Following this, measure the length of the segment on the paper. Using the calibration bar, duration (in second) of the segment can be calculated. For achieving frequency value, divide the number of the waves with the duration of the segment. For example, according to the calibration bar, 25 mm on the paper represents 1 second duration. The length of the analyzed segment is 23 mm then its duration is 23/25 = 0.92 s. If the segment contained 10 waves then the frequency is 10/0,92 = 10,78 Hz.

If the recording is dominated by beta waves, it is not necessary to calculate frequencies and amplitudes. However, it is still important to note the presence of the beta activity in the table!

If artefacts are present in the recording, define and mark their start and end points. Artefacts should be numbered and the numbers should be referenced in the table.

Compare the different EEG curves belonging to the different recording loci and then make approximations regarding the following aspects of the subject:

• awake or sleeping

• open eyes or closed eyes

• healthy or having some neurological problem

The healthy subject was in a seated position during the recordings with eyes closed. In case of the deviant recording/subject, name the supposed neurological disorder. Hypothesis should be explained in details. During the evaluation of the lab record, it is not the diagnostic accuracy that will be appreciated, but the careful and precise analysis of the recordings.

Chapter 11. Analysis of behaviour and learning

11.1. Introduction

Human and animal behaviour are regulated by genetically determined, inherited and plastic, learnt elements. Behaviour is the range of actions and mannerisms made by the animal or happening to it (moving, relaxing, sleeping, quiet wakefulness, regarding etc.). Behaviour is continuous and it also involves the lack of visible movements. Comportment represents a higher level of behaviour; it is characterized by foresight and intention and has aware (or deliberate) components.

Inherited regulatory mechanisms only, however, would not be able to adapt to the continuously changing surroundings. Therefore, adaptive functions, such as learning and memory, are needed in order to continually adjust behaviour to the requirements determined by natural and social environment. The inherent, overwhelming learning capacity of the brain enables us to recognise and understand the different tasks of different environmental challenges, and learn/produce the most appropriate answers. Inherited and learnt behaviour, therefore, are present together, they can only be distinguished by special methods.

Description of behaviour might be carried out by various methods. Usually, distinct behavioural elements are defined; their prevalence, duration and order can be recorded. Several techniques were established for the analysis and interpretation of experimental data, however, no widely accepted quantitative method is known. There are several ways for the characterization of behaviour. Before applying a method, we have to take into consideration the main purpose of the study, the involved species, and their natural inherited abilities. The available methods can be divided into two groups; the first group contains methods for testing the spontaneous activity of animals. In this group several subgroups can be defined:

1. Tests of spontaneous behaviour: the basic activity and patterns of spontaneous activity are recorded (i.e.: open-field test, motimeter).

2. Preference tests: Due to a pleasant or unpleasant stimulus, the animals prefer or avoid one part of the experimental box or a previously conditioned taste, movement pattern (i.e.: place-preference test, taste aversion, etc.).

3. Distress-test: these methods are based on a conflict situation. Stimuli, places, tastes, etc. might generate distress in experimental animals, while other stimuli, due to curiosity, motivation factor, reward, drive the animal to the preferred situation (i.e.: elevated plus-maze, run latency (fear reaction) test, Vogel thirsty-lick conflict test, etc.).

4. Social distress or social competence tests: these methods are based on social interaction of two or more animals. (i.e.: resident-intruder paradigm, social deprivation, etc.)

5. Motivation tests: the motivation level of individuals is measured (i.e.: Porsolt forced swim test, novel-object test, etc.).

The second group of experimental methods involves learning and memory tests:

1. Labyrinth tests: they always offer choices for the individual; the test might be a 'go - no-go' type straight alley, or a labyrinth with single or multiple junctions (straight alley, T- or Y-maze). In some forms of labyrinth tests, the central part of the experimental box is connected to several alleys, making the investigations of memory possible (i.e.: Radial maze), or due to the experimental conditions the spatial orientation can be studied (i.e.: Morris water maze).

2. Associative learning paradigms: the main purpose of these studies is the association of two stimuli during a conditioning process. This category involves contextual fear conditioning paradigms, operant conditioning, etc. (i.e.: Skinner-box).

Behavioural tests are mostly used in pharmacological research. Almost every aspect of behaviour and learning (spontaneous activity, distress, learning, memory and motivation) can be affected by psychoactive drugs; however

one might keep in mind that the alteration of behaviour can be also the consequence of changes in motor activity. For this reason, it is strongly recommended to perform motor activity tests (i.e.: rotarod, beam walking), to exclude the presence of confounding effects.

Unfortunately, the in vivo effect of a drug candidate compound cannot be predicted from the structure of the molecule. Therefore, several derivatives of the prospective drug compound are synthetized during drug research in hope to find the efficient version that has no serious side-effects. The large number of molecules to be tested, makes the selection of the promising ones difficult. Therefore fast screening procedures were developed to exclude those molecules which have no effect on expected behaviour or learning paradigms or cause adverse symptoms.

11.2. Investigation of spontaneous behaviour under different experimental conditions.

A sudden, new stimulus triggers automatically a pre-defined serial of behavioural patterns, even if the stimulus originates from the animal's natural surroundings. The first component is the startling reaction, when the animal „freezes" for a while due to the unknown situation. The secondary answer is the explorative reaction, during which sensory attention is turned towards the stimulus in order the explore it and to determine its significance. The stimulus can be either „attractive", tempting for closer examination, or can be „repellent", frightening, and leading to escape. The significance of the explorative reaction is clear: the animal must find out the most suitable behavioural answer to the altered situation. Fear and anxiety are the 2 strongest inner drives behind exploration, but curiosity can also participate to a certain extent. Understanding the basic „choreography" of orienting and explorative behavioural elements is indispensable during behavioural tests.

The goal of the experiments: to investigate the spontaneous behaviour and movement choreography of the rat, analysing its reaction for unexpected stimuli.

Required materials: open-field box, "Behaviour" software, rats

11.2.1. The open-field test

The open field must be illuminated evenly, and surrounding noise should be minimized. The field is divided into numbered squares (5x5 quadrants), which can be used to follow the animal's movement inside the experimental field. Different type of quadrants can be distinguished: central, peripheral and corner quadrants. Since illuminated, open fields induce distress in rodents, they will spend significantly longer time in peripheral and corner quadrants.
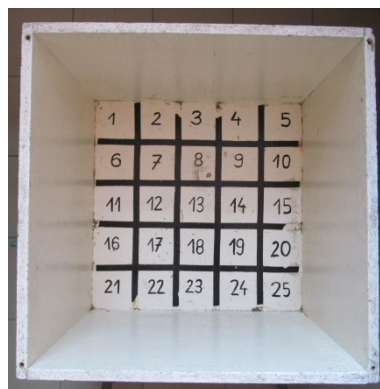
Figure 11.1. *The open field box*

Method: A laboratory rat should be placed in the middle of the field, and its activity and behaviour must be examined during a 5-minute interval. Recording of spontaneous exploratory activity of the rat should be done with the "Behaviour" software. The order and duration of basic behavioural elements can be recorded by clicking on buttons designated to the following elements:

• walking (W)

- sniffing (S)

- rearing (R)

- quiet (Q)

- moving on the spot (M)

- grooming (G)

In order to record spontaneous behaviour, start Behaviour software by double clicking on its desktop icon. Type the ID of the experiment and the ID of the rat into the given fields. Data will be stored in a text file, its filename generated by the concatenation of experiment and rat IDs. The recording session can be initiated by clicking the Start command in the Menu bar. In the pop-up dialog, after confirmation of filename and file location, recording can be triggered by clicking on 'Save' button. By clicking on buttons representing each behaviour element, spontaneous activity of the rat can be stored. The counter in the middle shows the elapsed time. Stop the recording after 5 minutes by clicking on 'Stop' command in the Menu bar.

The pattern of spontaneous behaviour might be visualized by generating a transition matrix or a flow-diagram. The transition matrix is composed of the number of transitions among two coupled behaviour elements. Pairs are generated in every possible combination resulting in a matrix of transitions. The flow-diagram composed of six circles, each representing a behaviour element. The diameter of the circles is related to the incidence of the given element. Arrows between circles show the direction of transition while their thickness is related to the incidence of that transition. Flow diagram can be generated by a Microsoft Excel macro, named "Calculator.xls". To use this, the text file should be imported to MS Excel as a delimited text file. Set the delimiter to comma "," then the whole dataset should be copied to the "A2" cell of Calculator.xls workbook. If the workbook contains data, these must be deleted previously. Run 'calc' macro by selecting 'Tools/Macros/Macro…' command. The macro will calculate the data of the transition matrix and create a flow-diagram.

In case of walking, the trajectory can be followed by observing and writing down the number of the quadrants visited by the animal within the box.

11.3. Unexpected stimuli, effect of social interaction.

Method: After recording the spontaneous behaviour of the rat in the open field for 5 minutes, start the next 5-minute long session to record the behaviour of the rat, while sudden unexpected stimuli (flashing light, sharp noise) are given. Compare the pattern of activity with the results of the first – control – session.

Finally place another (naive) rat into the box. Follow the behaviour of both animals for an additional 5 minutes, as indicated above. Compare the behavioural elements of the two animals, depending on whether it was the new one, or the one which already had the opportunity for exploration. Also record and analyse in your lab report, how the animals reacted towards each other. Discuss whether any new elements appeared in the behaviour of either of the animals.

Discussion: Analyse the main characteristics of the animal's behaviour in the two experimental conditions, including the most frequent behavioural elements and the main walking trajectories! Where did the animal spend the most time during the first and second half of the experiment? Did you observe any changes between the different behavioural elements, depending on the length of time the animal spent in the box? Try to explain your findings! Please include the flow diagram as attachment to your report.

11.3.1.Fear reaction

A special open field is used for this test. The field is circle-shaped and is attached to a small wooden box with a removable top. This test is suitable for measuring fear and anxiety.
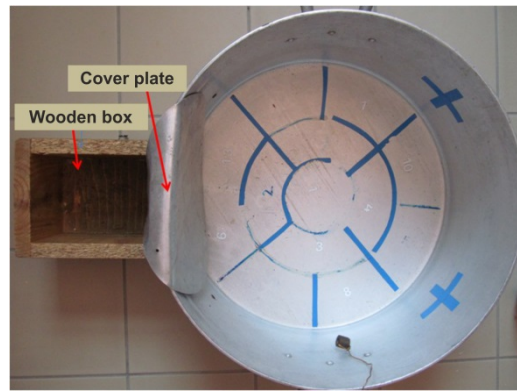
Figure 11.2. The round open field box

Method: Place a rat in the middle of the field. The rat will show some exploratory behaviour and then will run into the wooden box (which is dark and secure for the animal). Note the latency of running into the box. Record the behavioural elements and the pathway of the animal for 2 minutes, including the number of entering/quitting from the box. After 2 minutes, move the animal back into its home cage. Repeat the first step 2 times. Compare the latencies.

Evaluation: create a flow-diagram for each experimental session; latency for entering the box and the pathway of the rat should be also indicated. Try to find frequently occurring stereotypic patterns.

11.4. Y-maze test

The Y-maze is composed of 3 similar arms, which are distinguished by differently coloured spots at their ends. Rodents are very good at learning and sensing different directions, which can be tested by this simple equipment. The main task is to teach a thirsty rat to learn the correct direction (left side or right side) in order to get a drop of water.

Method: Clean the labyrinth with ethanol, and decide which direction you want to teach to the rat (e.g. right side). Place one drop of water at the end of one arm, then place a thirsty rat in the middle. The rat will run immediately into one of the arms. Once it happens, close the arm behind the animal with the plastic plate. If the rat runs into the arm which contained the water, let it consume. If not, wipe off the drop of water, then place a new drop of water into that arm which is now to the right to that arm where the rat run into. Once it is ready, lift the plastic plate and let the rat enter another arm. In case the rat enters now the arm to the right, let it consume the water. If the rat chose the left arm or goes back into the original arm, close the way behind it, wipe off the water and start again by putting a drop of water into the arm located to the right of the animal. Repeat the whole procedure until the rat learns that it has to choose a certain direction in order to get the water.

Figure 11.3. The Y-maze

Discussion: In your lab report, include the individual choices made by rat, from one session to the other. Give the time needed for teaching the correct direction! Once the animal learned the task, try to change the direction and observe how long it takes for the animal to get accustomed to the new requirements.

11.5. Operant conditioning

In operant conditioning, the animal makes a connection between its behaviour and the consequences that is either reward or punishment. The consequences of the behaviour have a regulatory effect on the behaviour: if the consequences are positive for the animal, the incidence of the given behavioural element will increase. In the classical (Pavlovian) conditioning, the animal is a passive participant. In contrast, in operant conditioning the animal is required to learn a task or solve a problem get the reward, or avoid punishment. As the animal achieves that by operating on its environment, this experimental method is called operant or instrumental conditioning (it was introduced by Thorndike in 1898.) At the practical, the main task is to teach a thirsty rat that it has to push a pedal in order to get some water.

Method: The equipment is a box (Skinner box), with a pedal in the front that the rat can easily press by its paw. Pressing of the pedal closes a contact that turns on a small lamp informing the experimenter about the event. Next to the pedal, there is a lever equipped with a small spoon immersed in a water-filled petri dish. By manually raising the lever, the experimenter can give a few tenth of millilitre water to the rat.

Place the animal into the box and put on the cover to reduce disturbing lights. Let the animal explore the new surroundings for a couple of minutes. Record the number of any spontaneous pushes on the pedal. After a few minutes, try to draw the animal's attention to the pedal, and also, try to make it understand that water can be reached from the spoon. Once the animal knows where to obtain the water from, try to teach the animal to make a connection between pushing the pedal and getting some water. If the animal fails to connect the task with the reward, you can try to put a drop of water directly onto the pedal. Be patient, teaching can take up to 1 hour!!! (This experiment measures not only the ability of the rat for operant conditioning, but also your ability/patience for teaching!)
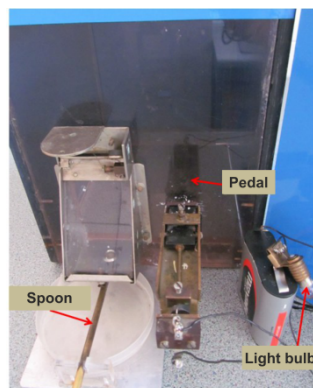
Figure 11.4. *The Skinner box*

Discussion: Include your observations and impressions during the test, and explain the rat's behaviour. Give the number of spontaneous pedal pushes and the duration of this period, the elapsed time between the beginning of experiments and successful conditioning.

11.6. Maze (labyrinth) test with hungry mice

Rodents are especially good in finding their way in tunnels, tubes, etc. Besides simple curiosity, hunger or thirst will motivate the animal even more to explore an unknown labyrinth. Strong motivation will increase the ability for learning, too. The behaviour is motivated by the known distant goal, and regulated by uncontrolled stimuli. More precisely the movements of the animal are regulated by the exploration of waypoints, texture of the labyrinth, while the food placed at the end point of the labyrinth enhances the motivation.

Method: Clean the inner surface of the labyrinth with ethanol in order to remove the smell of previous animals. Make a basic outline of the labyrinth in your lab book where you can also record the track of the mouse during the tests. Put some „reward" (sunflower seed, cheese, etc) to the exit in a small box.

Place a hungry laboratory mouse in a small box to the entrance of the labyrinth and record the way it makes through the labyrinth and also the time needed for the reaching the reward. Observe whether the animal entered some „dead-ends" and how long it took for it to recognize that it is a wrong way. Once the animal reaches the reward, do not let the mouse consume the whole piece (otherwise the animal will lose motivation to find the reward)! Place the animal back to the entrance and start the test again. Repeat the tests until the time needed for the complete run does not decrease any more.

Discussion: in your lab report, make a drawing of the labyrinth, and indicate the different routes made by the animal during the different testings. Give also the time needed to reach the reward during the consecutive tests. Could the animal find the shortest way to the food? Could you observe any alterations in the animal's behaviour during the consecutive tests? Was the animal able to learn the route and reach the reward fast?

Figure 11.5. *Labyrinth for testing spatial orientation*

Chapter 12. Investigating the effects of drugs on the central nervous system

12.1. Pharmacological studies

This chapter is about the pharmacology of the nervous system. Since pharmacology is not a compulsory course for students in Biology program, it is probable useful to shortly review the basic elements of this discipline, and some general issues related to preclinical and clinical research of nervous system drugs before proceeding to the detailed overview of the various classes of pharmacological agents. In the first part of this section, we discuss shortly how drugs reach their site of action inside the body, what factors can influence their action, how these processes can be characterized quantitatively, and what unwanted side effects may be associated with drug administration.

12.1.1. Quantitative aspects of drug effects

One possible goal of pharmacological studies is devising an efficient treatment regimen. During this process the quantitative relationship between the applied drug and its pharmacological effect is analyzed by constructing/plotting the so-called dose-response curves. Various drug effects can be analyzed at different levels, i.e. at subcellular, cellular, tissue levels or at the level of the whole organism. Drugs exert their effects in individual animals, and biological systems are variable therefore the response to a pharmacological agent may be different in different subjects. Thus, it is important to investigate drug effects in a sufficiently large number of animals. In spite of inter-individual differences in drug sensitivities the mean values can give a suitable approximation how a given subpopulation of experimental animals will respond to a drug treatment. Results obtained in a group of 4-8 animals are usually enough for performing statistical analysis. In many cases, the nature of the response is all-or-none, i.e. an effect either appears in an animal, or not, e.g. seizures, or lethality, in other cases the size of the responses can be quantified by a measured variable, such as changes in the velocity of nerve conduction or in the concentration of a transmitter. Typical semi-logarithmic dose-response curves have a sigmoid shape, although their middle portion is nearly linear. Doses belonging to the inflection points of these curves, namely the ED_{50}, and LD_{50} values, respectively, are used to help the comparison of the pharmacological and toxicological effects of different drugs. A drug at its ED_{50} dose cause a 50 % increase or decrease in a measurable variable compared to its maximum effect when investigated in a large population, or causes a pre-defined drug effect in 50% of the subjects if it is an all-or-none effect. Similarly, a 50% lethal effect is expected at the LD_{50} dose of the drug. The term "terapeutic window", refers to the ratio of the ED_{50} and the toxic dose TD_{50}, TD_{50}/LD_{50}; it indicates the safety of the drug. The wider is this window the less harmful is the drug. If the window is narrow, there is a higher chance for drug-overdose toxicity problems. The "therapeutic index" (TI) is the ratio of LD_{50} and ED_{50} ($TI = LD_{50}/ED_{50}$).

12.1.2. Drug application

To exert a therapeutic effect, the drug has to be present at a sufficiently high concentration in the organ affected by the disease. A drug is usually not evenly distributed in the body; it is advantageous if its concentration is the highest at the target organ. This fact should be taken into consideration when administering a drug; it is important to select the ideal route of application. Application can be local by using ointments, lotions, gels, skin patches, or aerosols allowing for the drug to reach a high concentration at the site of action without causing systemic side-effects. Systemic administration of a drug can be enteral, when tablets or capsules are taken through the mouth (per os; PO), or parenteral. The latter includes application of sublingual tablets, rectal suppositories, or different types of injections, and is mainly used when a drug brakes down at the acidic pH of the stomach, or is prone to enzymatic degradation in the intestines or the liver (first-pass metabolism). By injecting a drug, either via subcutaneous (SC), intramuscular (IM), intraperitoneal (IP), or intravenous (IV) route, the whole quantity of the drug gets into the blood stream, while enteralr routes provide a gradually increasing drug plasma level, the time course of which is largely determined by the absorption rate of the drug.

12.1.3. Absorption, therapeutic window

Drugs are transported to their site of action via the circulation; and to exert a therapeutic action they have to reach a critical concentration in the target organ. With the exception of the IV administration, pharmaceutical agents need to be absorbed, which may involve crossing several biological barriers, membranes. This generally occurs with passive diffusion or active transport, but sometimes involves filtration, or endocytosis. The rate at which an agent enters the circulation depends on the area of the absorbing surface, the diffusion distance to be covered, the duration of exposure, the velocity of local blood flow, the concentration gradient of the drug at the two sides of the biological barrier, its solubility, dissociability, and the ambient pH. Passive diffusion of a drug through a membrane is determined by its lipophilicity, which is characterized by its oil/water (octanol/water) partition coefficient. Lipid soluble molecules cross biological membranes readily by dissolving into the lipid-rich environment of the membranes. Most medicines are taken orally (PO). Mucous membrane of the oral cavity is relatively thin and is suitable for absorption. Furthermore, the ambient pH is near-neutral, which keeps most drug molecules (which are usually weak acids or weak bases) in their undissociated, lipophilic/apolar form, which helps their passage through the membrane. On the contrary, the extremely acidic pH of the stomach helps absorption of acids, but prevents that of bases, which are fully dissociated under these conditions. Conversely, the low-pH environment in the stomach can decrease the blood level of some drugs already in the circulation, since their distribution between the stomach lumen and the lumen of the blood vessels supplying the stomach (where the pH is neutral, and the molecules are diffusible) is shifted due to the continuous protonation, which decreases the concentration of diffusible molecules. Most orally applied agents, however, are absorbed from the intestines, which represent a much larger absorption area. While drug molecules absorbed from the oral mucosa as well as those from the rectum get directly into the systemic circulation, molecules absorbed from the intestines are collected by the portal veins of the liver, and may undergo rapid enzymatic degradation/metabolism. The extent of this first-pass metabolism can be so large that only insignificant amount of the drug reaches the blood stream after passing through the liver. Non-cooperative patients (unconscious people, small children) are frequently treated by suppositories, which are absorbed easily, and also avoid first-pass metabolism by the liver.

Absorption from the intestines is gradual, contributing to the sustained maintenance of the therapeutically efficient plasma level of the drug. If a retracted absorption is advantageous, it can be helped by choosing appropriate vehicles, crystal formulation, intestinosolvent tablets or capsules. In order to exert an appreciable therapeutic effect the drug must reach a certain plasma concentration, which depends on its potency. Increase of the dose further results in a dose-dependent elevation of its blood level and a more efficient treatment. Very high concentrations, however, may cause unwanted toxic effects. As described above, it is essential to determine the therapeutic window of a drug, in which dose/concentration range it exerts a significant therapeutic action without the risk of serious side effects. In many pathological situations (chronic diseases) a sustained (for months or even years) therapeutic plasma level is required. A more-or-less steady state blood concentration can be built up and maintained by repeated dosing.

12.1.4. Bioavailability

Bioavailability of a drug is calculated as a ratio of the amount of the dug that got into the circulation and that had been actually dosed. In the case of IV administration this figure is 100 %, while in the cases of other routs incomplete absorption and metabolism will decrease this value. For example if 60 mg appears in the circulation following PO administration of 100 mg, the bioavailability of the drug is 60%. Plasma concentration of a drug can be easily determined by taking a venous blood sample from one of the arms. Bioavailability is largely dependent on how rapidly the drug gets into the liver, and what the extent of its hepatic biotransformation is. Compared with PO applied agents, which are mainly absorbed from the intestines and get quickly into the liver, an IM administered drug gets first into the systemic circulation, and metabolized by the liver slower. Solubility of pharmacons is a critical factor regarding the absorption and membrane penetration. A considerable portion of poorly soluble substances remains in the lumen of the intestines and removed by the feces. Water soluble molecules are highly hydrophilic, and cannot penetrate through the lipid-rich membranes. Extremely large, hydrophobic molecules are not ideal as drugs either, since they cannot dissolve into the blood plasma and will bind to transport and other proteins. Furthermore, they are generally easily metabolized by liver enzymes. Technological formulation of pharmaceuticals is an important aid to compensate for poor absorption of compounds and to increase their bioavailability. Selecting an appropriate salt of the drug molecules, additives that help dispergation, the right vehicles, as well as using capsules or pills that are disintegrated in the proper site of absorption can all help the drug to reach a high plasma concentration. Two drug molecules with similar potencies on a target are regarded as biosimilar if their bioavailabilities are nearly

equal. Therapeutic equivalence means that the two drugs have similar efficacies, and they are also similar concerning safety aspects.

12.1.5. Distribution

Following their absorption drugs are carried to different parts of the body via the bloodstream, and get into the interstitial space by diffusing out from the blood vessels. Then a reversible process called distribution occurs, ie. the molecules are transferred between various extracellular and intracellular compartments. The drug is dissolved in a hypothetical volume (*volume of distribution*), which is the sum of the water volume in the blood plasma, interstitial space and intracellular space, although the exact definition and quantitative determination of these volumes is not an easy task. Extravasation of a drug from the plasma to the interstitium and its accumulation there mainly depends on the blood flow rate, permeability of capillary walls, binding to plasma and tissue proteins, and biophysical properties of the drug determined by its chemical structure. Great differences can be observed between the various tissues regarding the speed and amount of blood passing through a unit volume. Perfusion rates are relatively high in the brain, liver and kidney. The amount of drug entering the intersticial space from the blood vessels is largely determined by the structure of the capillary wall. In order to get into the brain drugs have to pass the blood-brain barrier. Lipophilic agents penetrate relatively easily by being dissolved in the lipid membrane of the capillary endothel cells, while those having a polar character or being ionized are practically not penetrating. Lipid solubility is important regarding the penetration through any biological membrane, such as that of intestinal epithelium, non-brain type capillary endothel, or neuronal plasma membrane. The extent of penetrability is influenced significantly by binding to proteins in the plasma, or interstitium. It is important to determine the ratio of protein-bound and free drug concentrations in the plasma. A decrease of the level of the free (dissolved in the plasma) drug level helps dissociation from the proteins, since the binding is an equilibrium process. Drugs are not trapped in one particular water space, they usually distribute among several compartments. The volume of distribution is an important parameter of a drug. It is calculated so that the total amount of the drug in the body is divided by its plasma concentration. Determining the time course of changes in plasma concentration following a rapid drug application provides information about distribution. It is important to take into account, however, that simultaneously with the process of distribution excretion of the drug will also begin, which influences the calculation of the volumes significantly.

12.1.6. Drug metabolism, elimination half-life

Absorbed foreign molecules are generally metabolized rapidly by the organism, and their metabolic transformation leads mainly to the formation of molecules with improved water solubility. Transformation takes place dominantly in the liver, but the role of epithelial cells of the intestines, lung and kidneys is also significant. The rationale of increasing water solubility is that hydrophilic molecules are excreted more easily through the kidneys. Several non-microsomal and microsomal liver enzymes that participate in drug metabolism are also known from regular metabolism. The speed of these reactions can be either lower or higher following repeated application of certain drugs (enzyme inhibitors and inducers, respectively). Elimination half-life ($t_{1/2}$) is an important characteristic of therapeutic agents; it is defined as the time necessary for the drug concentration to decrease by 50% from a steady-state level. $T_{1/2}$ depends on the volume of distribution and the whole-plasma clearance, and can be directly calculated from the elimination rate in the case when the concentration declines exponentially.

12.1.7. Excretion, elimination kinetics

Removal of drugs from the body, similarly to other molecules, occurs mainly through the kidneys. Elimination of the drugs (already transformed to water-soluble molecules) by the kidneys depends basically on the extent of glomerular filtration, on the rate of passive diffusion, and sometimes also on active tubular secretion and tubular reabsorption. Besides the kidneys the lung and some glands may have important role in excretion. Glands might excrete substances on the skin surface, or into body fluids, such as the bile. The lung does not possess any specialized excretion mechanism; gaseous and volatile molecules enter the alveolar space according to their partial pressure, and then exhaled. Drugs excreted into the bile or saliva not necessary leave the body; they can be reabsorbed later in the gastrointestinal tract.

Excretion is not the only way to eliminate drug effecst. Metabolic transformation can result in the loss of pharmacological efficacy. Strong binding to certain tissues may remove some molecules rapidly from the plasma; the

molecules getting accumulated in the adipose tissue, liver, spleen, etc., and can be stored there for long periods while providing only low plasma levels.

12.2. Drug developmental aspects

Compounds found efficient in the initial steps of drug research have to pass a long series of preclinical and clinical examinations before being registered and marketed as a new drug. It is inevitable that the drug candidate meets serious toxicological and teratological requirements; it also should not induce allergic reactions, mutagenicity or any tissue damage. After being found effective and safe in all *in vitro* and *in vivo* preclinical experiments, human clinical studies with the drug candidate can start, first involving healthy volunteers. This first phase may reveal some intolerable human-specific psychotropic side effects, which is an important issue in the case of central nervous system agents. In the next phase, the clinical studies may be continued on properly selected groups of patients.

12.2.1. Altering brain functions by chemicals

Normal functioning of the brain can be changed by applying *drugs, medicines* in various ways. *Neuropharmacology* is a discipline that studies molecules having neuronal effects, especially those that can be used in the therapy of neurological disorders. In contrast, *Psychopharmacology* focuses on drugs affecting psychological functions and psychiatric diseases. Data obtained in neuro- and psychopharmacological studies, however, can be utilized by neurophysiologists when investigating mechanisms governing brain functions and behavior, and these drugs provide useful methodological tools for their studies.

Below we give a short overview of the classification and possible mechanisms of action of nervous system agents in order to help a better understanding of the practical lesson dealing with pharmacological modulation of behavior.

12.2.2. Classification of agents acting on the central nervous system and their mechanisms of action

A *drug* is a substance that can change the physiological state of the organism. Many drugs – mainly lipophylic small molecules can get into the brain via crossing the blood-brain barrier, and are able to alter behavior by changing brain functions in various ways.

Some of the mechanisms by which they influence brain functions:

1. changing the general metabolic state of brain cells,

2. decreasing or increasing neuronal membrane excitability,

3. blocking action potential conduction along axons,

4. changing the blood supply of brain areas,

5. altering the distribution of water and ions between neurons and other brain cell types,

6. activating or blocking cell surface or intracellular receptors of transmitters; via these mechanisms central nervous system agents can alter normal brain function, and can normalize pathological brain functions caused by disases.

Grouping of neurotropic drugs according to their therapeutic actions:

1. General anesthetics (sometimes also called narcotics; see: surgery);

2. Opioid, or narcotic analgesics (e.g. morphine derivatives);

3. Non-narcotic, or minor analgesics (e.g. salycilates, anilin- or pyrazolon-derivatives);

4. Sedato-hypnotic drugs (e.g. barbiturates, benzodiazepines, urethane);

5. Convulsive agents, vasomotor/respiratory stimulants, or analeptics (e.g. pentylene tetrazole, picrotoxin, strychnine, caffein);

6. Anticonvulsant agents or antiepileptics (e.g. barbiturates, hydantoine- and oxazole-derivatives);

7. Centrally acting muscle relaxants (e.g. propanediol and benzoxazole-derivatives);

8. Antiparkinson drugs (e.g. dioxy-phenylalanine or dopa, atropine, some antihistamines).

A large group of agents act by altering higher brain functions, thus having an ability to influence psychological functions. Classification of these drugs is based on the knowledge of brain chemistry and of the elements of behavior. Main classes of *psychoactive* or *psychotropic* agents are the following:

1. *Psychostimulants* – Enhance brain functions, have an arousing effect, and decrease appetite (e.g. *amphetamine*, fenfluramine).

2. *Central nervous system depressants* – A group of agents called minor tranquilizer (benzodiazepines, barbiturates, alcohol) weaken or slow down neuronal activity and induce sleep or coma at high doses; they inhibit learned and unlearned behavior as well.

3. *Antipsychotics* – Decrease or eliminate hallucinations, psychomotor excitement and aggressivity of schizophrenic patients, and help the accomodation to the social environment of these socially passive patients. Via inhibition of central nervous system dopamine receptors they increase the excitability of hypothalamic and limbic structures. Some representatives include *phenothiazines,* such as chlorpromazine, and *butyrophenones* such as haloperidol.

4. *Antidepressants* – mainly used for treating mood disorders (depression). They inhibit reuptake of biogenic amines, such as tricyclic antidepressants (*imipramine),* or inhibit monoamine oxidase (MAO). In contrast to drugs against schizophrenia, where suitable animal models are available for drug testing, no appropriate model exists for depressions.

5. *Sedato-hypnotics* – Also called minor tranquilizers and anxiolytics, but the same drug also induces sleep at high doses. Alcohol and barbiturates – as well as meprobamate have several side effects. Benzodiazepines *(e.g. diazepam or chlordiazepoxide*), similarly to the former agents, enhance GABAergic transmission, but have better safety profile.

Studying drug effects on animal behaviour (behavioural pharmacology) is a very important discipline within medical research. Besides helping research and development of new drugs it supplies important information about functioning of the brain and the regulation of behavior. Its findings may be important also for ethologists. On the other hand results of etology may be valuable for scientists involved in drug research e.g. by suggesting new study methods. It is important to keep in mind, however, that drugs showing reasonable efficacy and side effect profile in animal experiments can be completely ineffective, or toxic in patients, and vice-versa, agents successfully applied to treat human diseases may be without effect in animals (e.g. meprobamate).

Central nervous system active agents are classified based on the results of various assays, including behavioral and biochemical pharmacological tests. Simple behavioral tests, such as the Irwin observation test battery are usually performed on small rodents. They are commonly used to evaluate the effects of new substances on central nervous system's functions, and are suitable for rapid screening of new compounds with potential therapeutic efficacy. Vegetative symptoms are also recorded to get information on the potential side effects of the test drugs concerning the autonomic nervous system.

The *Irwin* observation test battery is widely used for assessing the risk of drug-induced toxicity. Activity of adult male mice is observed in different test situations as described below. Drug treated animals are observed individually and their behavior is scored in each test on a 0-8 point scale. The scores are compared with that of the control animals. Differences between control and treated animals in each test are summarized on score profiles, where score differences of the individual tests are displayed grafically. Score values and their differences from control scores will indicate if the studied drug a) has a central nervous system action, b) if it has, then what kind of action it is.

12.3. Rapid screening of central nervous system active drugs

Aim of the practical lesson: Introduction of the simplified *Irwin observational test battery*, used for studying drug effects on behaviour. Effects of both behavioral stimulant and behavioral depressant (tranquilizer) agents will be analysed and demonstrated. At the beginning of the lesson a short review on the main characteristics and mechanisms of action of the drugs to be studied (some common, easily available compounds) will be given.

Course of the practice: Experiments will be performed on mice pretreated with the test agents before the start of the lesson. In order to avoid the excessive disturbance of the animals during observation, the animals are held in cages larger than their usual home-cage. Test procedures and the basic principles of evaluation of drug effects are described below.

Examinations will be based on simple visual obsevation of behavioral abnormalities, and an 8-point arbitrary, subjective scale will be used for evaluation of drug effects. When the Irwin test battery is used for scientific purposes, drugs are tested by experienced observers, a large number of animals is used and each particular observation is repeated several times allowing reliable conclusions. Naturally, only a much smaller number of animals can be used in the present practical lesson. Gross behavioral differences between control and drug treated animals and alterations in their physiological status are scored. For a particular element of behaviour, a score of 0 is assigned to normal behavior, when it is not present in control animals, while a score of 4 when it is observable in controls and the drug can cause a deviation to both directions.

First the behavior and physiological state of control animals should be observed thoroughly.

1. *Urination* – control: 0

2. *Salivation* – control: 0

3. *Exploration* (exploratory behavior in a novel environment) – control: 4. Normally, the animal is sniffing around, circumambulate (the edges of) new objects, etc. If it does not start exploring activity, or shows reluctance to do so, the score should be decreased; if it does with abrupt, swift movements, vehemently, or for a disproportionately long time tenaciously and persistently, then the score should be increased.

4. *Level of alertness and vigilance* – control: 4. sedation or sleepiness means smaller, while excessive arousal and alertness means bigger score.

5. *Grooming (self-cleaning) – control: 4. – Frequently observable in normal animals as well.*

6. *Spontaneous activity in the cage* – control: 4. sleeping or numb animal: 0, continuous walking: 6, walking with running: 7, excited bursts of running: 8.

7. *Restlessness* – control: 0. Continuous mouving

8. *Stereotype behavior* – control: 0 Characterized by repeating a particular element of behavior such as circling, head nodding or twitching, tail flicking, etc. The type of movement should be noted as well.

9. *Vocalization* – control: 0. Normally, resting animals do not vocalize.

10. *Change in skin color(head and tail)* – control: 4. Skin color is normally pink, or slightly reddish. Some drugs cause paling others cause flushing.

11. *Piloerection* – control: 4. Drugs may sometimes cause an erection of the fur accompanied by wetting caused by salivation.

12. *Torment* – control: 0. Suffering

13. *Tremor* – control: 4. Most frequently affects the trunk and limbs.

14. *Spasms and convulsions* – control: 0. Repetitive unvoluntary muscle contractions of limb flexors (clonic seizures), with or without whole-body involvement (tonic seizure).

15. *Trepidity*– control: 0.

16. *Body posture* – control: 4. Lower score is assigned when the animal is dragging its abdomen on the floor, or cannot use its limbs properly. The score is higher in the cases of hunching or gibbosity, torsion, extended body during walking.

17. *Limb positionand tension* – control: 4. If the drug causes and abduction of the limbs lower scores are given, while when the position of the limbs is closer to the trunk compared with control animals the score will be higher.

18. *Abnormal walking, ataxia* – control: 0. Tottering, unsteady, ataxic gait, dragging the trunk will increase the score.

The next step is carried out by extending our hand toward the animals in the observational cage without touching them. The following reactions should be noted:

19. *Irritability* - – control: 0. Appearance of agressiv behaviour.

20. *Wincing response to sound* – control: 4. Control animals display moderate response to loud clapping of hands.

21. *Pain reaction* – control: 4. Normal animals flick their tails when pinched with a pair of tweezer. Weak or lack of reaction will decrease, while a fierce response increase the score.

22. *Cornea reflex* – control: 4. Speed of the eye closure.

23. *External ear reflex* – control: 4. Following touching shake of outhwr ear

24. *Flexor reflex* – control: 4. Normally, pinching of the toes will evoke the withdrawal of the affected hindlimb (ipsilateral flexion reflex). Drug effect may result in the decrease or lack of flexor response (lowest score), or increased, generalized (to other limbs, or the whole body) response (high score).

25. *Eyelids closure* – control: 4. Normally, the eyes are open, or open to slight stimulation. Constantly drooped eyelids mean lower, while constantly open eyes mean higher scores. *(weakened blink-reflex?)*

The next step is to carry out hand-held observations. Grab the skin on the neck of the animals and place them on the table.

26. *Goggling eyes*

27. *Fear reaction* – control: 0. Normally, mice tolerate simple manipulation without attempting to flight or fight fiercly. Such reactions increase the score.

28. *Reactivity, motility* – control: 4. Low walking activity: 1, continuous ambulance: 6, walking mixed with running periods: 7, wild/excited running: 8.

29. *Placing reflex* – control: 4. The animal is held by its tail, approached to the surface of the table and finally slowly placed on the table. Normal animals pre-set their limbs in the proper position, thus the legs will firmly lean on the surface in the right sequence at landing. Lack of the reflex will result in low scores, while its spastic, rigid, or fierce accomplishment will result in high scores.

30. *Passivity* – control: 0. The walking mouse is grabbed at the neck. A normal mouse will try to escape; if it is fighting only when lifted up vertically: 2, if it fights only when it is placed into a suppine position/on its back: 4, if it fights only when its forelimbs are touched: 6, if it (does not fight, or) fights only when it hindlimbs are handled: 8.

31. *Grip strength* – control: 4. The animal is positioned on a grid by its tail, or a thin wooden stick is placed to its forelimbs. Then we try to remove the animal or the stick by pulling either the animal or the stick away. Normally,

the animal will resist for some time, then it gives up. If it cannot hold itself, or its grip is weak, it receives a low score; if its grip is stronger and firmer that usual, and holds itself with tensed muscles, it receives a high score.

32. *Body tension – control: 4.*

33. *Limb muscle tone – control: 4. Normally, limbs will resist against flexion by contraction of the extensor muscles. If the limb muscles are atonic, flaccid, it will result in a low score, if the resistance is high, the muscle contraction is tense and strong the score will be high.*

34. *Inclined plate test – control: 4.there is no control score value. The animal is placed on a metal plate equipped with a bevel. The plate is slowly lifted up at one of its edges, so that 90 degree is reached in 5 seconds. The degree of inclination when the animal begins to slip down is recorded, rather than a score value.*

The score sheet for testing central nervous system activity of drugs can be found in the Appendix.

Preparation of lab report:*Score values* are to be recorded and shown in a *table* form. Score profiles are also to be displayed graphically (*profile-figures)*. On this figure, results for the individual tests are shown as points. The y coordinate of the points indicates the serial number of the test, while the x coordinate is determined by the score value obtained. By connecting these points we get a profile that can be compared with the similarly generated profile of an other drug. This type of presentation is not suitable for statistical analysis.

Compare the effects of the different agents studied, and try to characterize their behavioral effects!

Chapter 13. Polygraphic recording and biofeedback

13.1. Introduction

The polygraph (Greek-derived word, poly=many, graph=write), which is commonly referred to as a lie detector, records physiological activities (blood pressure, pulse, respiration, and skin conductivity) associated with arousal in the autonomic nervous system. Although the most well-known application of polygraphy is in criminology, a number of medical instruments (e.g. patient monitor units used in intensive care) can be also regarded as polygraph.

One of the underlying principles involved in using the polygraph as a lie detector is that the autonomic nervous system control the heart rate, respiratory rate, blood pressure and flow, as well as sweating, and this control cannot be consciously altered. Another principle is that emotional changes associated with intentional falsification of answers to carefully selected and worded questions involuntarily and subconsciously alter autonomic output that cause recognizable changes in the recorded physiological variables.

During the practical, heart rate, respiratory rate, and sweating, i.e. **galvanic skin response (GSR)** are simultaneously measured by ECG electrodes, a respiratory transducer and electrodes measuring the conductance between two fingers. GSR is based on the fact that the human skin displays several forms of bioelectric phenomena, especially in areas of the extremities such as the fingers, palms of the hands, and soles of the feet. Emotional excitation during lying causes sympathetic activation. Heart rate, respiration frequency and perspiration will increase. Sweat glands are innervated by the sympathetic nervous system through cholinergic fibers. Sympathetic activation causes an increased secretion of sweat. The increased level of sweat on the skin decreases the galvanic skin resistance (GSR) and increase skin conductance (GSC) because of the ionic content of the sweat. Changes in GSR and GSP related to the emotional state of the subject constitute the galvanic skin response.

It is important to know, that changes in the measured responses not necessarily indicate deception, and, on the other hand, deception is not necessarily cause changes in the measured vegetative responses. The aim of the polygraph examiner is to establish a psychological set in the examinee that will increase the likelihood that any observed arousal to specific questions is the result of deceptive responses. Whether this arousal is caused by a fear of being caught with a lie, a conditioned response to the act of lying, orientation to a matter of emotional salience, the increased cognitive processing required for deception, or some other mechanism is unclear, although theories involving orientation to threat and emotional salience are becoming increasingly popular.

The most common type of polygraphic examination involves a pre-test interview, a small number of questions with "yes" or "no" answers that are asked while the examinee is attached to the polygraph, and a post-test debriefing interview that takes place after the charts have been evaluated. The whole process usually takes between one and three hours. Critics argue, however, that the autonomic reactions can be influenced by the subject (e.g. at inert questions increasing the muscle tone) and the reactivity is strongly depending on personality (e.g. anxious individuals may have strong responses to neutral questions).

There have been numerous studies addressing the accuracy of polygraphic tests; some were based on laboratory designs such as mock crimes, others on reviews of its use in field settings. Reports of accuracy are in the region of 70-90 percent.

Biofeedback training is a learning process whereby people learn to exert conscious control over physiological processes normally controlled by the autonomic nervous system. The biofeedback instruments noninvasively monitor physiological parameters (e.g. blood pressure, heart rate) and provide a feedback signal to the subject in real-time. The subject can use the received signal to enhance the desired response. The biofeedback training allows the subjects to be able to regulate certain autonomic processes: control headaches, lower blood pressure, lower heart rate and manage responses to stressful situation. In the practice, biofeedback training has been shown to be effective for controlling hypertension. For example, in "warm hand" training the biofeedback signal is associated with the temperature of the palms. The subject is instructed to warm up his/her hands and due to the significantly increased blood flow due to the reduced peripheral resistance, the blood pressure decreases.

The measured variables can be fed back to the subject by several ways. The variables or signals created from them – e.g. the heart rate converted to sound, or a motion of a pointer – can be displayed to the subject, and his/her task is to increase or decrease its value. In another method, the success of desired change is fed back but not its value. In the practice, the former method has not been really successful. Due to the time delay, the subject has received feedback from an earlier state; therefore changing the behavior was difficult. In the therapy, the second method is used.

13.2. Human polygraphy and biofeedback training

Aim of the practice: To analyze the effects of emotions on the autonomic physiological variables measured by the polygraph.

Used materials: BIOPAC MP30/35/36 acquisition unit with respiratory transducer (SS5LA), electrode lead set (SS2L) and galvanic skin response electrodes (GSR, SS3LA)

13.2.1. Experimental methods of polygraphy

During the measurement, three channels are used. The subject's respiration, heart rate and skin responses are monitored by a chest belt (respiratory transducer), ECG and galvanic skin response sensor (GSR), respectively. Prior to the experiment, attach the respiratory transducer around the chest below the arms and above the nipples (Figure. 13.1). The respiratory transducer must be slightly tight at the point of maximal expiration. If the tension is correct, simply attach the Velcro ends together. For the best results, the belt should be used over thin clothing.
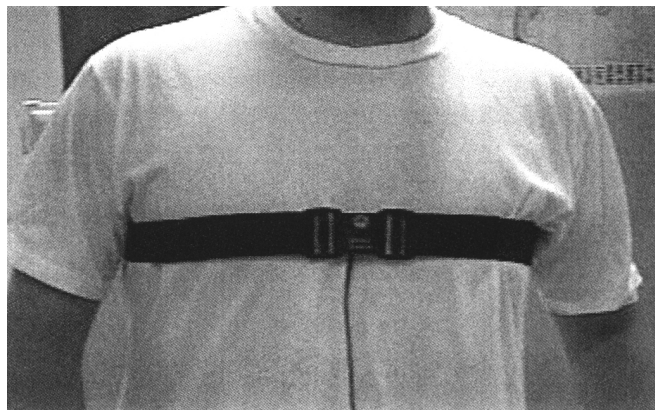
Figure 13.1. *Correct placement of the respiratory transducer.*

You must fill each cavity of the GSR electrodes with electrode gel to obtain accurate recordings. The SS3L electrodes should be attached to the fingertips of the index and middle fingers on the left hand of the subject, at least five minutes before the beginning of the recording (Figure. 13.2A). The sensors are positioned on the button of the fingertips and the Velcro tape is wrapped around the finger to fix the electrodes firmly.
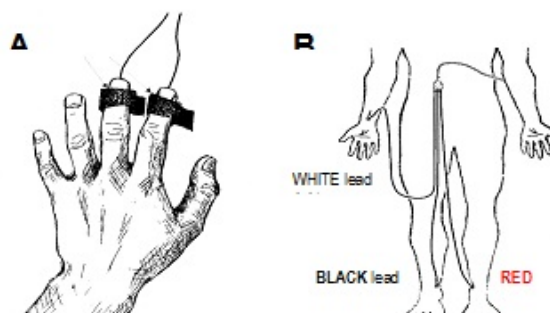
Figure 13.2. Correct attachment of GSR (A) and ECG (B) electrodes.

The disposable ECG electrodes should be placed on the legs and the right arm as shown on Figure 13.2B and the electrode lead set should be attached to the electrodes according to Einthoven's Lead II (Figure. 13.2 B).

Make sure, that the Biopac MP30/35/36 acquisition unit is OFF when you connect the cables to the device! The respiratory transducer should be connected to the first channel (CH1), the lead set to the second channel (CH2) and the GSR sensor to the third channel (CH3) as shown in Figure 13.3. Turn on the Biopac unit! Run the BSL 3.7.6 program and select Lesson 9: GSR & Polygraph from the pop-up list!
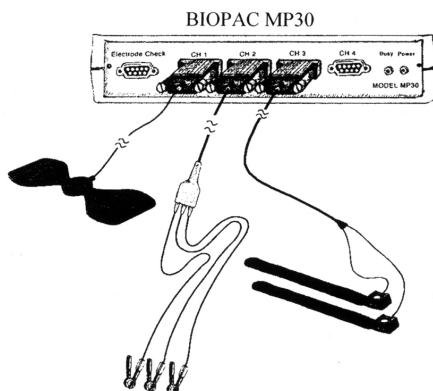
Figure 13.3. Equipment connections

At the beginning of data acquisition, a *calibration* procedure optimises the amplification of the signals by the hardware. After clicking on the **[Calibrate]** button, follow the instruction of the pop-up windows. If the calibration procedure fails, it can be repeated by **[Redo calibration]** command. If calibration was correct, the measurement can be started with the **[Continue]** command.

13.2.2. Experimental methods of biofeedback experiment

In the biofeedback measurement, the same electrodes and leads are used as in polygraphy. To set up the equipment, **make sure, that the Biopac MP30/35/36 acquisition unit is OFF!** Plug the GSR lead to the first channel (CH1) and the ECG lead set to the second channel (CH2) as shown in Figure 13.4. (The respiratory transducer is not used in this experiment.) Turn on the Biopac unit! Run the BSL 3.7.6 program and select Lesson 14: Biofeedback from the pop-up list!

At the beginning of data acquisition, a *calibration* procedure optimises the amplification of the signals by the hardware. After clicking on the **[Calibrate]** button, follow the instruction of the pop-up windows. If the calibration procedure fails, it can be repeated by **[Redo calibration]** command. If calibration was correct, the measurement can be started with the **[Continue]** command.
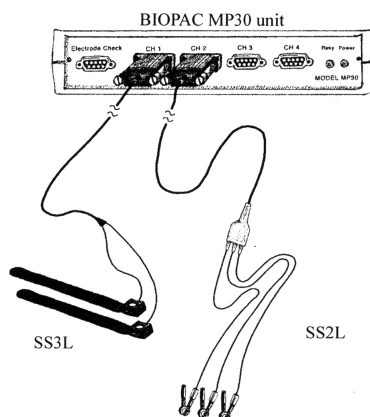
Figure 13.4 *Correct equipment set-up*

Experimental tasks

I. Polygraphy

Important aspects at each task:

- You should wait at least for 10-20 sec after each question/stimulus. Time is needed to see unequivocal changes in the recording. *The exact time of the stimulus should be marked in the recording by pressing the **F9** function key!*

- Pause the registration between tasks by pressing the **[Suspend]** button! The registration can be continued by pressing the **[Resume]** button.

- The subject is not allowed to see the recording (if he/she can see it, then it is called biofeedback).

- Completely silent enviroment is needed for the experiment. The observer is not allowed to show any reaction to the subject's answers, because it can influence the subject.

In the initial, "setup" phase of the measurement, the following scheme should be followed:

1. Subject has to tell his/her name.

2. Subject has to count back from 10 to 1.

3. Subject has to count back from 30 to 1 by subtracting increasing odd numbers (i.e. 3, 5, 7, etc.) from the previous number.

4. A person (preferable from the other gender) should gently touch the face and the hand of the subject.

The words/questions used in the following tasks should be constructed before the experiments!

5. Read out ten words to the subject. A 20 sec long pause between the words is needed. Choose words that should not evoke emotional reactions, like table, spoon, etc., and words which are expected to generate increased activity, like kiss, mother, subject's name, etc. *The associative (Jung) test is carried out similarly, investigating reactions to words that might have some "deeper" meaning, like father, death, flower, fire, etc. The subject's reaction profile might be used as a diagnostic tool.* However, reactions strongly depend on the subject's previous experiences. In extreme cases, it is possible that, for example "coat hanger" triggers strong emotions in someone due to bad childhood memories.

A possible list of words (those marked by an asterisk might elicit increased activity from most people).

Toothbrush

Ignition key

Bookmark

Scalpel*

Table

Love*

Coffee-spoon

Coat

Mother*

Calendar

Name of the subject*

Cup

Hypodermic needle*

Salt dispenser

Display

Kiss*

6. The subject has to answer ten "yes or no" type questions. The subject is instructed to lie once. Timing of the question and the answer must be marked by pressing F9 function key! At least 20 sec should elapse between questions!

A possible list of questions:

Were you born in Budapest?

Do you have brown hair?

Do you study psychology?

Do you have a sibling?

Have you ever travelled on a helicopter?

Do you watch the American TV-series „Emergency"?

Do you have a girlfriend (boyfriend)?

Do you like spinach?

Have you been in a theater in the last month?

Did you give true answers to all questions?

7a. Prepare 10 cards, write a number in increasing order on each of them. The subject should pull a card without showing it to anyone. The card should be put back, the cards should be shuffled, and then the investigator should show the cards one after the other in a random order and ask whether it was the card the subject pulled. The subject should say NO in all cases, and the others should try to figure out, which was the false answer based on the polygraphic recording. Wait at least 20 seconds between each question!

7b. Repeat the test, but now the subject has to show the drawn card to a third person, who is present during the course of the test.

II.Biofeedback

1. Subject is instructed to decrease his/her heart rate and galvanic skin resistance (GSR) without watching the screen. (Closed eyes, deep breathing, relaxed state, imagination of a nice place.)

2. If the previous exercise is successful, subject is instructed to try it with opened eyes. Important: audience should not comment the events as this can decrease the performance of the subject for minutes. (This exercise is paradoxical: when a person is asked to reach a relaxed state, the pressure to achieve the goal will work against relaxation.)

3. If the previous exercise is successful, subject have to try to increase his/her heart rate and GSR and then to decrease. This will only be successful in case of „talented" subjects.

Data analysis

Open the recorded file (Lessons/Review Saved Data/Name of the subject folder/<…>-L9 file). Set the screen by the Zoom tool and by the Autoscale waveforms option (located in the Display menu) to see the signals adequately. Print the screens of the record from the start to the end (4-6 sheets).

Mark the questions/exercises on the printed sheets! Report the changes (i.e: when do you see activation, when did the subject lie based on the changed physiological parameters, etc.) Try to locate when the subject gave deceptive answers! Compare the received results with the report of the subject!

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Appendix A.

Descriptions of the used physical, chemical and mathematical units, concepts and procedures

The observed processes in the physiological experiments are largely based on physical and chemical laws. The knowledge of the basic physical and chemical units is important to understand the measurement procedures and to evaluate and interpret the results. The mostly used units and concepts are collected in this section.

Units

In the most cases, units used in the physiological measurements correspond to the **SI** (Système International d'Unites) **standard** system, which is based on a few selected units and the powers of 10. However, there are also some tolerated, non-SI units such as mmHg (used to express the pressure of the body fluids) or liter (L) as the unit of volume. Base units of SI are the followings:

Unit	Symbol	Dimension	Dimension symbol
Meter	**m**	length	**l**
Kilogram	**kg**	mass	**m**
Second	**s**	time	**t**
Ampere	**A**	electric current	**I**
Kelvin	**K**	temperature	**T**
Mole	**mol**	amount of substance	**n**
Candela	**cd**	luminous intensity	$\mathbf{I_v}$

The base units are precisely defined autonomous units. All other units of the SI system are derived by combining, multiplying or dividing base units (for example, force unit N (newton) = $kg \times m^{-2} \times s$).

SI prefixes can be assigned to the units, to denote decimal multiples. The prefixes are the powers of 10:

Prefix	Symbol	Factor	Prefix	Symbol	Factor
exa	E	10^{18}	deci	d	10^{-1}
peta	P	10^{15}	centi	c	10^{-2}
tera	T	10^{12}	milli	m	10^{-3}
giga	G	10^{9}	micro	μ	10^{-6}
mega	M	10^{6}	nano	n	10^{-9}
kilo	k	10^{3}	pico	p	10^{-12}
hekto	h	10^{2}	femto	f	10^{-15}
deka	da	10^{1}	atto	a	10^{-18}

Prefixes are used with base units and the units derived from them, for example. mN = 10^{-3} N.

Physical units and concepts

Volume

The non-SI *liter* (L or l) is often used as a unit of *volume* for liquids and gases:

$$1\,\text{L} = 10^{-3}\,\text{m}^3 = 1\,\text{dm}^3$$
$$1\,\text{ml} = 10^{-6}\,\text{m}^3 = 1\,\text{cm}^3$$
$$1\,\mu\text{l} = 10^{-9}\,\text{m}^3 = 1\,\text{mm}^3$$

Force

The **force** equals mass times acceleration ($F = m \times a$). **Weight** is a special case of force as weight equals mass times acceleration of gravity ($F = m \times g$). The force is expressed in newton (N) = $\text{kg} \times \text{m} \times \text{s}^{-2}$.

Pressure

The **pressure** equals force per unit area, so the SI unit of pressure is $\text{N} \times \text{m}^{-2}$ = Pa (pascal). A frequently used non-SI pressure unit is the mmHg, the pressure of 1 mm high mercury column (1 mmHg = 133.3 Pa).

Frequency

In the practice, repetitive biological phenomena are frequently investigated. In this case, it may be necessary to measure the elapsed time between events (time periods) or the frequency of an event. **Frequency** is used to describe how often a periodic event (heart beat, breathing, etc.) occurs per unit time. The SI unit of frequency is 1/s or Hertz (Hz), but 1/min is also commonly used (eg, heart rate is expressed in beats/minute [bpm]).

The frequency (f) and time period (t) of a repetitive event reciprocally define each other, therefore, $f = 1/t$ (for a given time unit).

Electrical Units

The flow of electric charge in a conductor is called **current**. The charge is carried by electrons, ions or so-called positive holes. Amount of particles flowing per unit time is specified as **current intensity** (I) and measured in ampere (A).

Electrical current cannot occur unless there is an **electrical potential difference** (voltage difference, U) between the two points of the electrical conductor. The potential is the energy that is needed in the electric field to bring a unit charge from the infinity into the point where the value of the potential is determined. The electrical potential (or voltage) is measured in volts (V).

According to the Ohm's law, the **electrical resistance** (R) of the conductor determines the electrical current that flows at a given potential difference: $R = U / I$. The electrical resistance is measured in ohms (Ω). $\Omega = \text{V} \times \text{A}^{-1}$

The **electrical conductivity or conductance** is the reciprocal of resistance ($1/\Omega$), measured in siemens (S).

In case of the so-called **direct currents** (DC), the net flow of charges occurs in one direction, whereas the direction of flow of **alternating current** (AC) changes at a given frequency (for mains current it is 50 Hz in Europe and 60 Hz in the USA).

Units used to measure the amount of light:

The **luminous flux** (SI unit: *lumen*) is the measure of the perceived power of the light. One lumen is defined as the luminous flux of light produced by a light source that emits one candela of luminous intensity over a solid angle.

Luminous intensity (SI unit: c*andela, cd*) is a measure of the wavelength-weighted power emitted by a light source in a particular direction per unit solid angle.

Illuminance is the total luminous flux incident on a surface, per unit area. It is a measure of how much incident light illuminates the surface. Its unit is *lux (lx)*.

Luminance is a photometric measure of the luminous intensity per unit area of light travelling in a given direction. It describes the amount of light that passes through or is emitted from a particular area, and falls within a given solid angle. The luminance expressed in *candela/m²*.

Units used to measure the sound waves

The **sound** is a sequence of longitudinal waves that are propagated through media such as air or water. Longitudinal sound waves are waves of alternating pressure deviations from the equilibrium pressure, causing local regions of compression and rarefaction. Sound waves are often simplified to sinusoidal plane waves, which are characterized by frequency (**the pitch**) and the amplitude of the wave (maximal differential pressure=**sound pressure**).

Sound pressure (SP) is the difference, in a given medium, between average local pressure and the pressure in the sound wave. **Sound pressure level (SPL)** or sound level is a logarithmic measure of the effective sound pressure of a sound relative to a reference value. It is expressed in decibels (dB) and shows the relation of the intensity of a given sound (I) to the intensity of sound with 20μPa sound pressure (I_0):

$SPL = 10 \log (I/I_0) = 10 \log (p/p_0)\ 2 = 20 \log (p/p_0)$.

(According to the laws of physics, the wave intensity is proportional to the square of the amplitude.) The decibel reference value was set to the sensitivity of the human ear, as the pressure of barely audible tone of 1000Hz is 20μPa.

Therefore $P = P_0$ SPL $= 20 \log (20μPa/20μPa) = 20 \log (1) = 0$ dB.

Chemical concepts and units

Concentration

Concentration expresses the amount of the solute in one unit of the solution. Concentration can be defined as:

• **Mass concentration**, or the mass of a substance per unit volume (e.g., g/L = kg/m^3)

• **Molar concentration**, or the amount of a substance per unit volume (e.g., mol/L)

• **Molal concentration**, or the amount of substance per unit mass of solvent (e.g., mol/ kg H_2O).

Osmolarity, osmolality

Osmolarity is the concentration of all osmotically active particles in a solution, regardless of which compounds or mixtures are involved. Its non-SI unit is Osm/L (osmol/L).

Since the osmolarity unit refers to volume, which depends on the temperature and volume of the solute, **osmolality** is used more often, refering to the weight of the solvent (Osm/kg H_2O). In practice, osmolality can be easily measured easily by osmometer.

pH

The **pH** indicates the H^+ ion concentration of a solution. pH is the negative common logarithm of the molal hydrogen ion concentration: pH =-log10 $[H^+]$.

Fast Fourier-Transformation

During the quantitative analysis of the EEG signal, recordings are divided to consecutive epochs with pre-defined duration (usually 4 – 20 seconds) then Fast Fourier Transformation (FFT) algorhythm is performed. FFT is a mathematical algorhythm which is used frequently in physics. Principle of FFT lies in the hypothesis that EEG waves appearing in the pre-defined epoch have different frequency, amplitude and phase values and can be described as a sum of sine and cosine waves. Result of the FFT shows quantitatively the amplitude values of waves belonging to different frequencies as well as phase information. FFT results in power spectra which shows numerically and expressively the frequency composition of the analyzed EEG epoch as well as dominant frequency or frequencies. Power spectra can be generated from any EEG recording. However, the original EEG signal can not be reconsctructed from the power spectrum as phase information disappears during the calculation of spectra from the transformed signal.

Determination of sensory thresholds

Sensory threshold is a theoretical concept used in psychophysics. A stimulus that is less intense than the sensory threshold will not elicit any sensation. Methods have been developed to measure thresholds in any of the senses.

Several different sensory thresholds have been defined:

- **Absolute threshold:** the lowest level at which a stimulus can be detected.

- **Recognition threshold:** the level at which a stimulus can not only be detected but also recognised.

- **Differential threshold:** the smallest difference between two stimuli that can be percieved.

- **Terminal threshold:** the level above which changes in a stimulus are no longer detected.

Thresholds measured in a given time depend on several factors. Actual arousal level of the central nervous system (i.e.: the patient is active or tired etc.) has a huge influence. Motivation is also a factor as well as the actual state of the sensory apparatus (i.e. intact or damaged). This means that thresholds are not constant, these parameters can change even in case of the same patient.

Methods used for determination of sensory thresholds:

1. **Method of limits**. In the first step, the subject is stimulated by strong, easily detectable stimuli that are decreased stepwise (**descending sequence**) until he/she can not detect the stimulus. Then another stimulation sequence is applied called **ascending sequence**. In this sequence, stimulus intensity increases from subthreshold to easily detectable. Both sequences are repeated several times. This yields several momentary threshold values. In the following step, mean values are calculated for ascending and descending sequences separately. The mean value will be lower for descending sequences. In case of audiometry, the difference of the means in case of ascending vs. descending sequences has a diagnostic importance. In the final step, average of the previously calculated means will result the absolute threshold.

2. **Method of constant stimuli.** Stimuli of **varying intensities** are presented **in random order** to a subject. Intensities involve stimuli which are surely subthreshold and stimuli which are surely suprathreshold. For the creation of the series, the approximate threshold judged by a simpler method (i.e.: by the method of limits). The random sequences are presented to the subject several times. The strength of the stimulus, percieved in more than half of the presentations will be taken as the threshold.

3. **Adaptive method**. Stimulation starts with a surely suprathreshold stimulus, then further stimuli are given with an intensity decreased in previously-defined steps. The series is stopped when the stimulus strength become subthreshold (this is the so called „turn" phenomena). Then the step is halved and the stimulation is repeated, but now with increasing intensities, until the subject perceives the sound again. This process is repeated several times, until the step size reaches the preset minimal value. With this method, the threshold value can be delineated very accurately. The initial size of the step can be selected depending on the expected accuracy.

Texts for the oculogram

Once upon a time, a young man by the name of Tom was walking through a park. The trees were just coming into leaf after a long harsh winter. Just ahead of him Tom saw an old man trip, then fall to the ground. He rushed to his aid, gently lifting him up and guiding him to a nearby seat. After regaining his breath, the old man thanked Tom.

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Once upon a time, a young man by the name of Tom was walking through a park. The trees were just coming into leaf after a long harsh winter. Just ahead of him Tom saw an old man trip, then fall to the ground. He rushed to his aid, gently lifting him up and guiding him to a nearby seat. After regaining his breath, the old man thanked Tom. From their seat they could see a broad river bounding the park on one side. A host of ships, great and small, were passing to and fro. The old man had once been a sailor and knew each ship by name.

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The glutamate receptor channels (GluRs) mediate most of the the fast excitatory synaptic transmission in the mammalian central nervous system. NMDA receptors (NRs) belong to a subtype of GluRs with some unique properties: NRs are physiologically blocked by Mg^{++} in a voltage-dependent manner and are highly permeable to Ca^{++}, pointing to the possibility of working as "coincidence detectors" in important physiological functions such as experience-dependent synaptic plasticity in the adult as well as developmental brain plasticity.

Il était une fois un jeune homme appelé Thomas. Un jour il se promenait dans le parc. Sur les arbres, des petites feuilles apparaissaient après un hiver long et très froid. Juste devant lui, Thomas vit un vieil homme trébucher et tomber. Il courut à son aide et gentiment il le releva et le conduit vers un banc en proximité. Le vieil homme remercia Thomas avec gratitude. De leur banc, il pouvaient juste voir une grande rivière bordant le parc. Beaucoup de bateaux, petits et grands y naviguaient. Autrefois, le vieil homme était marin, donc il connaissait le nom de chaque bateau.

Es war einmal ein junger Mann der hieß Tim. Einmal ging er spazieren in den Park. Auf die Bäume öffneten sich kleine Blätter nach einem langen und kalten Winter. Plötzlich sah Tim einen alten Mann bevor sich stolpern und hinfallen. Er rannte um ihn zu helfen und begleitete ihn zu einer Bank in der Nähe. Sobald der alte Mann wieder zu Atem kam, bedankte er Tim herzlich. Von ihren Platz konnten sie einen breiten Fluss sehen, wo viele Schiffe, kleine und große, hin und her fuhren. Der alte Mann war damals ein Seemann und er kennte jedes Schiff bei Name.

1. data report and evaluation: *Analysis of human blood* - determining blood group type and blood glucose level

Experiment was accomplished by:

....

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Teacher:

....

Date:

....

Subject's

name: .... height: ....

age: .... weight: ....

gender: male / female

Aims of the practical lesson *(brief definition in a few sentences)*

Introduction *(can be continued on a separate sheet if needed)*

1. Main characteristics of human blood (brief summary about the types and importance of human blood cells and about the composition of blood plasma; description of human blood types, their importance and characterisation) *(summary of the most important facts in regard to the analysis of the practical data)*

2. Methods and materials *(schematic enumeration)*

3. Technical details of the practical exercises *(preparation of the subject and the experiments, explanation of the exercises)*

XML to PDF by RenderX XEP XSL-FO Formatter, visit us at **http://www.renderx.com/**

1. Determination of the Subject's blood group type

Experimental results and evaluation

a) Make a schematic drawing of the experimental findings!
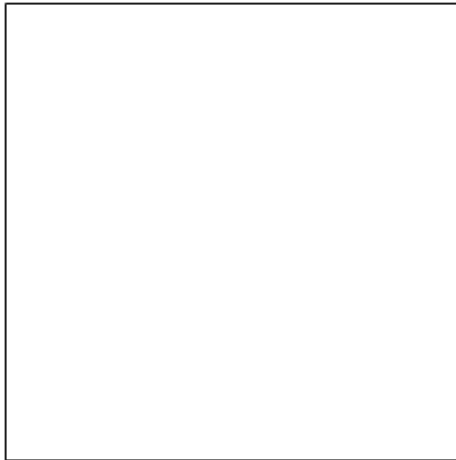
b) In which solution did you observe agglutination of the investigated blood?

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c) According to the above results, which blood group type does the investigated blood belong to? Give a detailed reasoning in your answer!

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d) To which blood group type patients can the investigated Subject donate blood? Why? Explain your answer!

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e) From which blood group type donors can the investigated Subject receive blood during a transfusion? Why? Explain your answer!

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e) In case a woman has the same blood group type as the investigated Subject. Is there a risk for developing Rh incompatibility reaction during her pregnancy? Give a detailed reasoning in your answer!

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2. Determining blood glucose level

Experimental results and evaluation

a) How high blood glucose level did the Subject have during the testing? Does it belong to the normal, pre-meal (or fasting) range?

Subject's blood glucose level: ....mmol/l

Normal pre-meal (or fasting) blood glucose level: ....mmol/l

....

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b) In case the measured blood glucose level differs from the normal, pre-feast value, give some possible explanation(s) for the observed differences!

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c) What kind of physiological symptomps accompany severe diabetes mellitus (Type 1 and Type 2 diabetes)? Why is it a life-threatening situation and how can it be treated?

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definitions

antigen: ....

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antibody: ....

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agglutination: ....

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anti-D: ....

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AB0 blood group type: ....

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Rh incompatibility: ....

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2. data report and evaluation: Electrocardiography - effects of body posture, breathing, physical activity and circulatory reflexes on heart function

Name of the students:

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Teacher:

....

Date:

....

Subject profile

Name: .... Height: ....

Age: .... Weight: ....

Gender: male / female

Goals of the practical lesson *(brief definition in a few sentences)*

Introduction *(can be continued on a separate sheet if needed)*

1. Heart function and blood pressure regulation (briefly); fundamentals of the ECG and blood pressure measurement *(summary of the most important facts in regard to the analysis of the practical data)*

2. Methods and materials *(schematic enumeration)*

3. Technical details of the practical exercises *(preparation of the subject, settings of the recording system, explanation of the exercises)*

Blood pressure measuring

Measure the systolic and diastolic blood pressure and pulse (BPM) and give them accordingly in the table! Calculate the pulse pressure and the arterial mean pressure!

(measured on the left arm)	systole [Hgmm]	diastole [Hgmm]	pulse pressure [Hgmm]	arterial mean pressure [Hgmm]	pulse [beat/min]
lying down					
sitting					
standing up					
after exercise, standing					

II. Examination of the ECG components

Data of Einthoven lead II. can be found as channel 2 (Ch2) in the recording. For the analysis of the given exercise, magnify a section in the recording and then select the appropriate measurement function in the measurement type box. In the area selected by the I-beam cursor, **ΔT** (delta time) measurement is the difference in time between the end and the beginning in the selected area (usually it equals to period time).

Measure and calculate the requested parameters during the consecutive cardiac cycles and then complete the tables below and depict the requested values on graphs. Pay attention to give the appropriate unit when filling out the tables. **Attach the magnified, analyzed recording segments to the lab record and note the number of the attachments.**

II/1. Analyze 3 consecutive cardiac cycles and measure the duration (**ΔT** – delta time) of the ECG components listed below. Select the start- and end points of the given ECG component by the I-beam cursor. Complete the table with the measured data and then calculate the mean values. Complete the last column of the table.

ECG Component	Duration (ΔT) [............] Cardiac cycles			mean	Which event of the heart cycle occurs during this component?
	1.	2.	3.		
P wave					
PQ segment					
QRS complex					
QT interval					
ST segment					
T wave					
TR segment					
Number of the attachment:.....................					

III. Normal sinus arrhythmia during deep inhalation-exhalation

III/1. Measure the duration (period time; **ΔT**) of the cardiac cycles during at least 2 consecutive inspiratory and expiratory epochs. Select the area between the consecutive R peaks by the I-beam cursor. Then calculate the mean and the difference between the maximal and minimal values (max-min). Fill the table with as many cardiac cycle data as belong to a single respiration epoch.

III/2.	Cardiac cycle								mean	max-min
	1.	2.	3.	4.	5.	6.	7.	8.		
inhalation I. ΔT [......]										
exhalation I. ΔT [......]										
inhalation II. ΔT [......]										
exhalation II. ΔT [......]										

III/2. Depict the durations of the consecutive cardiac cycles (period time, **ΔT**) on the graph below. On the graph, X axis shows the number of the consecutive cardiac cycles while y axis shows the period time (**ΔT**) of the cardiac cycles. Mark the onset of the inhalation and exhalation epochs on the graph.

III/1. Sitting, deep Inhalation / exhalation

period time [ΔT; sec]

number of consecutive heart cycles
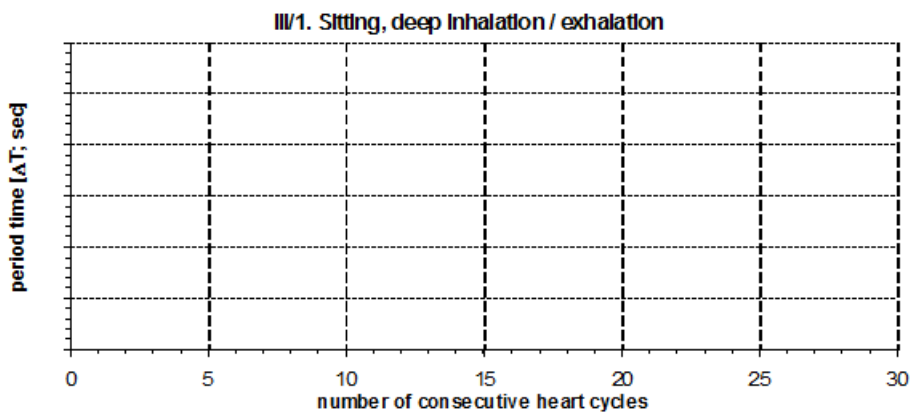
Number of the attachment:..................

IV. Effect of sudden stand-up (circulatory reflex)

IV/1. According to the instructions, ensure enough time for the recording of the supine state as well as for the state after the stand-up. Mark the time point of the stand-up on the attachment and on the graph.

Select the area between the consecutive R peaks by the I-beam cursor. Measure the duration of the cardiac cycles (period time, **ΔT**), then depict the values on the graph below. On the graph, X axis shows the number of the consecutive cardiac cycles, while y axis shows the period time (**ΔT**) of the cardiac cycles.

III/2.	Cardiac cycle								mean	max-min
	1.	2.	3.	4.	5.	6.	7.	8.		
lying down ΔT [......]										
time point of the stand-up (effect) ΔT [......]										
early aftereffect (first 5 cycles after stand-up) ΔT [......]										
late aftereffect (10th-15th cycles after stand-up) ΔT [......]										

IV/2.

VI/1. Effects of sudden stand-up
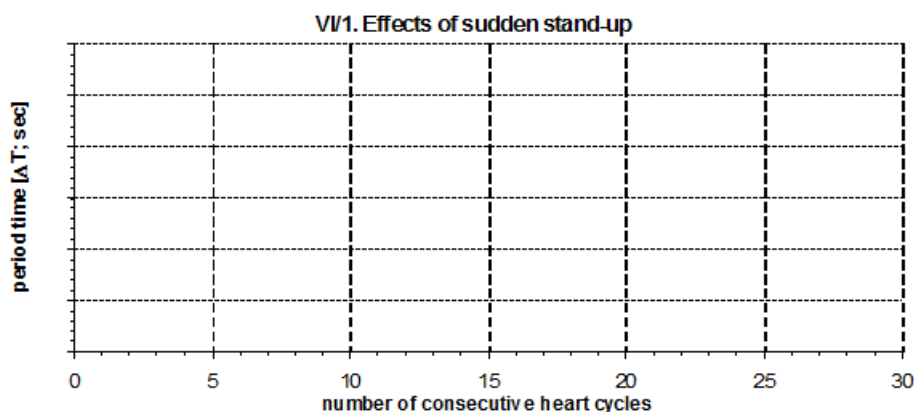
Number of the attachment:..................

V. Effect of physical activity

V/1. Compare the results obtained immediately before and after physical activity. Analyze 8 consecutive cardiac cycles and measure the duration of the cycles (**ΔT**□- period time). Select the area between the consecutive R peaks by the I-beam cursor. Calculate the mean of the individual durations and the difference between the minimal and maximum **ΔT** values (max-min). Calculate the heart rate in BPM (beat per minute).

V/1.	Cardiac cycle								mean	max-min
	1.	2.	3.	4.	5.	6.	7.	8.		
immediately before the physical activity ΔT [......]										
Number of the attachment:...................								BPM:...		
immediately after the physical activity ΔT [......]										
Number of the attachment:...................								BPM:...		

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V/2. Analyze 5 consecutive cardiac cycles and measure the duration (ΔT – delta time) of the ventricular systole (QT interval) and the ventricular diastole (TR segment - between the end of the T wave to subsequent R peak). Calculate the mean.

Number of the attachment:	duration (ΔT) [.............] Cardiac cycle					mean (ΔT) [.............]
	1.	2.	3.	4.	5.	
immediately before the physical activity						
QT interval (ventricular systole)						
TR segment (ventricular diastole)						
immediately after the physical activity						
QT interval (ventricular systole)						
TR segment (ventricular diastole)						

B. Data summary and conclusions

1. Effect of body postures

a) How did blood pressure and pulse rate change when the subject changed his/her body position?

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b) Give appropriate physiological explanations accounting for the above effects!

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c) Sudden change of the body position from lying to standing often causes dizziness or –mainly in the elderly - fainting. How can you explain this phenomenon?

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2. Effects of the respiration (normal sinus arrhythmia)

Summarize the results in the table below.

	Mean period time (ΔT) [............]
seated, deep inhalation	
seated, deep exhalation	

Does breathing influence heart frequency? Explain your answer.

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3. Effect of physical activity

Summarize the ECG results in the table below.

	BPM (mean)	QT interval (mean) [.......]	TR segment (mean) [.......]
immediately before the physical activity			
immediately after the physical activity			

How does physical activity influence the duration of the ventricular systole and diastole as well as the heart frequency? Describe the physiological background of the observed changes.

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How did muscle exercise influence arterial blood pressure and pulse rate? Systolic or diastolic blood pressure values were changed to higher extent upon the exercise? What is the explanation for this?

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According to the observed changes, was the subject well-trained for physical exercise? Explain your answer!

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4. Effect of sudden stand-up

Summarize the results in the table below.

	before the intervention (control)	during the intervention (effect)	during the first five cycles after the intervention (early aftereffect)	during the 10th-15th cycles after the intervention (late aftereffect)
ΔT (mean) [.........]		-		
BPM (mean)		-		

a) How does heart frequency change after sudden stand-up? Was there a difference between the first seconds of the stand-up (early aftereffect) and during the later period (late aftereffect)?

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b) Explain the function of the circulatory reflex specified at 4.a).

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C. Definitions

ECG:................. ───

Systole:..

Systolic   pressure:...

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D   i   a   s   t   o   l   e   :

....

Diastolic   pressure:..

....

Arterial   mean   pressure:..

....

Pulse   pressure:...

....

Respiratory   sinus   arrhythmia:...

....

Baroceptor   reflex:...

....

Peripheric   resistance:..

....

3. data report and evaluation: Blood pressure measurements - effect of body position and physical exercise

Experiment was accomplished by:

....

....

....

....

Teacher:

....

Date and time:

....

Subject's

Name: .... Height: ....

Age: .... Weight: ....

sex: male / female

Aims of the practical lesson *(brief definition in a few sentences)*

Introduction *(can be continued on a separate sheet if needed)*

1. Factors and physiological changes influencing human blood pressure *(summary of the most important facts in regard to the analysis of the practical data)*

2. Methods and materials *(schematic enumeration)*

3. Technical details of the practical exercises *(preparation of the subject, explanation of the exercises)*

A. Data and calculation

Measure the systolic and diastolic blood pressure and pulse (BPM) and give them accordingly in the table! Calculate the arterial mean pressure!

	systole [Hgmm]	diastole [Hgmm]	pulse (beat/min)	Arterial mean pressure [Hgmm]
measured on the left arm:				
lying down				
sitting				
standing up				
after exercise				
measured on the right arm:				
sitting				

B. Summary and discussion of data

1. Effects of body position

a) How did blood pressure and pulse rate change when the subject changed his/her body position?

....

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b) Give appropriate physiological explanations accounted for the above effects!

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c) Sudden change of the body position from lying to standing often causes dizziness or –mainly in the elderly - fainting. How can you explain this phenomenon?

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2. Comparison of blood pressure and pulse rate values between the left vs. right arm

a) Did blood pressure values differ in case measurement was carried out in the left or in the right arm? Why? Give a physiological explanation the observed findings!

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b) Did pulse rate values differ in case of left or right arm measurements? Why? Explain the observed findings!

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3. Effects of physical exercise

a) How did muscle exercise influence blood pressure and pulse rate? Systolic or diastolic blood pressure values were changed to higher extent upon the exercise?

....

....

....

....

b) According to the observed changes, was the subject well-trained for physical exercise? Explain your answer!

....

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....

C. Definitions

systole: ....

systolic pressure:..

....http://www.renderx.com...........................

diastole: ....

diastolic pressure: ....

....

arterial mean pressure:..

....

4. data report and evaluation: Investigating human respiration (spirometry) - analysis and comparison of the pulmonary functions

Experiment was accomplished by:

…………………………………………..

…………………………………………..

…………………………………………..

…………………………………………..

Teacher:

…………………………………………..

Date:

…………………………………………..

__Aims of the practical lesson__ (brief definition in a few sentences)

__Introduction__ (can be continued on a separate sheet if needed)

__1. Brief summary about the regulation of human respiration__ (summary of the most important facts in regard to the analysis of the practical data)

__2. Methods and materials__ (schematic enumeration)

__3. Technical details of the practical exercises__ (preparation of the subject, settings of the recording system, explanation of the exercises)

Subjects' profile:

1st subject

Name: …………………………………………........

gender: male / female

age:……………………………………………………..

height:……………………………...................

weight:………………………………………………..

smoker?:………………………………………..

active sportsman or dancer?:……………..………

asthmatic?:………………………………………......

2nd subject

Name: …………………………………………........

gender: male / female

age:……………………………………………………..

height:……………………………...................

weight:………………………………………………..

smoker?:………………………………………..

active sportsman or dancer?:……………..………

asthmatic?:………………………………………......

3rd subject

Name: …………………………………………........

gender: male / female

age:……………………………………………………..

height:……………………………...................

weight:………………………………………………..

smoker?:………………………………………..

active sportsman or dancer?:……………..………

asthmatic?:………………………………………......

4th subject

Name: …………………………………………........

gender: male / female

age:……………………………………………………..

height:……………………………...................

weight:………………………………………………..

smoker?:………………………………………..

active sportsman or dancer?:……………..………

asthmatic?:………………………………………......

5th subject

Name: …………………………………………........

gender: male / female

age:……………………………………………………..

height:……………………………...................

weight:………………………………………………..

smoker?:………………………………………..

active sportsman or dancer?:……………..………

asthmatic?:………………………………………......

6th subject

Name: …………………………………………........

gender: male / female

age:……………………………………………………..

height:……………………………...................

weight:………………………………………………..

smoker?:………………………………………..

active sportsman or dancer?:……………..………

asthmatic?:………………………………………......

A. Data and analyses

1. Static measurement of pulmonary volumes

Fill out the tables below with the data of the recorded respiratory volumes and capacities! **Attach the analyzed records (printouts) to the lab report!**

1.1. Compare static respiratory data between a woman and a man with approximately identical body size:

	Short subject (name:..)		Tall subject (name:..............................)	
	Measured values [.......]	Percentage of the reference value [%]	Measured values [.......]	Percentage of the reference value [%]
IVC				
IRV				
ERV				
TV				

1.2. Compare static respiratory data between a short and a tall subject with identical gender:

	Short subject (name:..)		Tall subject (name:..............................)	
	Measured values [.......]	Percentage of the reference value [%]	Measured values [.......]	Percentage of the reference value [%]
IVC				
IRV				
ERV				
TV				

1.3. Compare static respiratory data between a healthy and a smoker/asthmatic subject with identical gender and body size:

	Short subject (name:..)		Tall subject (name:..............................)	
	Measured values [.......]	Percentage of the reference value [%]	Measured values [.......]	Percentage of the reference value [%]
IVC				
IRV				
ERV				
TV				

2. Dynamic measurement of pulmonary volumes

Fill out the tables below with the data of the recorded respiratory volumes and capacities! **Attach the analyzed records (printouts) to the lab report!**

2.1. Compare dynamic respiratory data between a woman and a man with an approximately identical body size:

	Woman (name:……………..…………….)		Man (name:…………..…………….)	
	Measured values [.......]	Percentage of the reference value [%]	Measured values [.......]	Percentage of the reference value [%]
FVC				
FEV$_1$				
FEV$_1$/FVC				

2.2. Compare dynamic respiratory data between a short and a tall subject with identical gender:

	Woman (name:……………..…………….)		Man (name:…………..…………….)	
	Measured values [.......]	Percentage of the reference value [%]	Measured values [.......]	Percentage of the reference value [%]
FVC				
FEV$_1$				
FEV$_1$/FVC				

2.3. Compare dynamic respiratory data between a healthy and a smoker/asthmatic subject with identical gender and body size:

	Woman (name:……………..…………….)		Man (name:……………..…………….)	
	Measured values [.......]	Percentage of the reference value [%]	Measured values [.......]	Percentage of the reference value [%]
FVC				
FEV$_1$				
FEV$_1$/FVC				

B. Data summary and conclusions

a) Mark with a relation sign in the table which of the subjects had higher measured respiratory volumes! Indicate the expected relation in the *reference* or *theoretical value* line and the recorded data below (*measured value*)!

Comparison of the respiratory values between a man and a woman			higher / smaller (< / >)	
IVC	*reference*			
	measured value	woman		man
TV	*theoretical value*			
	measured value			
FEV$_1$/FVC	*reference*			
	measured value			

Comparison of the respiratory values between a man and a woman			higher / smaller (< / >)	
IVC	*reference*	woman		man
	measured value			
TV	*theoretical value*			
	measured value			
FEV$_1$/FVC	*reference*			
	measured value			

Comparison of the respiratory values between a man and a woman			higher / smaller (< / >)	
IVC	*reference*	woman		man
	measured value			
TV	*theoretical value*			
	measured value			
FEV$_1$/FVC	*reference*			
	measured value			

b) How can you explain the difference in the measured values between a man and a woman? Give a detailed reasoning in your answer!

....

....

....

....

c) In which respiratory values did you observe differences between short and tall subjects? How can these be explained?

....

....

....

....

d) In which type of respiratory values are you **not** expecting differences if you are compare respiratory values between healthy and smoker/asthmatic subjects? Explain your answer!

....

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....

....

e) Rank the examined subjects into an order by using the characteristic reference IVC values (1.: largest, 6.: smallest)! Also give the order of the analyzed subject by using the measured IVC values!

Examined subjects	IVC order	
	Reference	**Measured value**
	1.	
	2.	
	3.	
	4.	
	5.	
	6.	

Are the two lists equal? If not, how can you explain the differences?

………

………

f) Bronchodilatator drugs open up airways and clear mucous. How would this affect the pulmonary function? Which respiratory volumes will change and how after the intervention?

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g) If somebody has chronic inflammation of the lungs (pneumonia), which respiratory volume will be affected in the diseased person?

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C. Definitions

vital capacity:……………………………………………………………………………………………..

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dynamic respiratory volume:……………………………………………………………………………..

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spirometer:………………………………………………………………………………………………...

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FEV1:……..

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………………………………………………………………………… http://www.renderx..…………

FVC:……

……

……

tidal volume:…………………………………………………………………………………………………

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5. data report and evaluation: Electromyography (dynamometry) - motor unit recruitment and fatigue

Experiment was accomplished by:

....

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....

....

Teacher:

....

Date and time:

....

Subject 1

Name: ....

Age: ....

Sex: male / female……………………

Height: ....

Weight: ....

Subject 2

Name: ....

Age: ....

Sex: male / female……………………..

Height: ....

Weight: ....

The aim of the experiment *(brief definition in a few sentences)*

Introduction *(can be continued on a separate sheet if needed)*

1. Regulation and adjustment of muscle contraction strength *(summarize those regulatory processes which are important during the data analysis and evaluation)*

2. Materials and methods *(schematic enumeration)*

3. Description of the experimental design *(preparation of the subject, settings of the recording system, explanation of the exercises)*

A. Data and calculation

1. Motor unit recruitment

Fill out the corresponding tables using *Segment 1* data both from the dominant and from the non-dominant arm. During the evaluation, choose the following options in the channel windows: **CH1 - Force**; **CH3 - Raw EMG**; **CH 40 - Integrated EMG**. Set up the following measurements as follows: **CH1: mean**; **CH3: p-p**; **CH40: mean**. Using the I-beam cursor, select an area on the plateau phase of the first clench, read the values and insert them into the table. Repeat the same order with the subsequent peaks - you may not need 9 peaks to reach maximum clench force. **Attach the analyzed records (printouts) to the lab report and number them accordingly!**

1. Subject 1 (name:...)

	Dominant forearm (right left)			Non-dominant forearm (right left)		
	number of the attached graph:........................			number of the attached graph:........................		
	Force at peak [CH1] mean [kg]	raw EMG [CH3] p-p [mV]	Integrated EMG [CH40] mean [mV]	Force at peak [CH1] mean [kg]	raw EMG [CH3] p-p [mV]	Integrated EMG [CH40] mean [mV]
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						

2. Subject 2 (name:...)

	Dominant forearm (right left)			Non-dominant forearm (right left)		
	number of the attached graph:........................			number of the attached graph:........................		
	Force at peak [CH1] mean [kg]	raw EMG [CH3] p-p [mV]	Integrated EMG [CH40] mean [mV]	Force at peak [CH1] mean [kg]	raw EMG [CH3] p-p [mV]	Integrated EMG [CH40] mean [mV]
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						

Create a **graph** of the observed changes in the **muscle strength (Force at peak) - integrated EMG** data pairs! On the x axis, indicate mean force measured at the peak of the subsequent clenches and give the corresponding integrated EMG amplitude on the y axis (determine the y axis scale according to the measured EMG values). Include data from both subjects and both forearms on the same graph by using different color for the 4 sets of data!

XML to PDF by RenderX XEP XSL-FO Formatter, visit us at **http://www.renderx.com/**
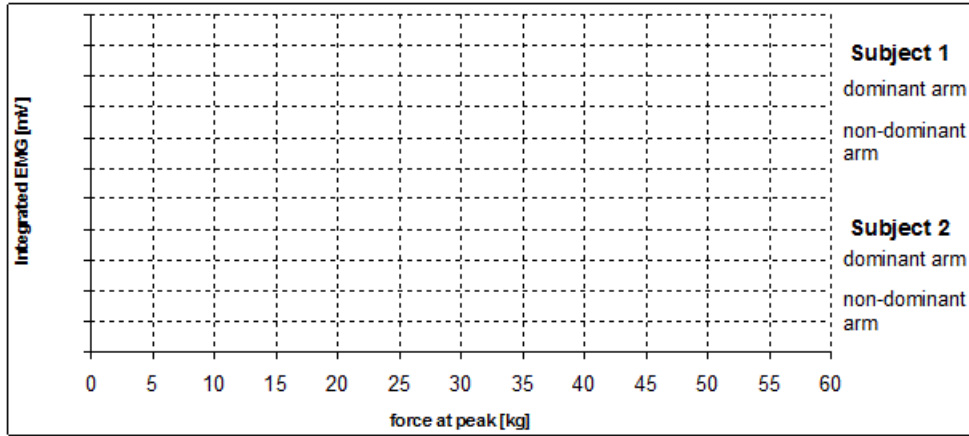
2. Fatigue

Complete the tables using the *Segment 2* data from each arm. Set up the measurement boxes as follows: **CH1: mean**; **CH40: mean**; **CH40: delta T (ΔT)**. **Attach the analyzed records (printouts) to the lab report and number them accordingly!**

1. Subject 1 (name:...)

	Maximal strength		50% of maximal strength		Fatigue length [CH40] ΔT [sec]
	Force at peak [CH1] mean [kg]	Integrated EMG [CH40] mean [mV]	Force at peak [CH1] mean [kg]	Integrated EMG [CH40] mean [mV]	
Dominant forearm (left right) record:............					
Non-dominant forearm record:............					

2. Subject 2 (name:...)

	Maximal strength		50% of maximal strength		Fatigue length [CH40] ΔT [sec]
	Force at peak [CH1] mean [kg]	Integrated EMG [CH40] mean [mV]	Force at peak [CH1] mean [kg]	Integrated EMG [CH40] mean [mV]	
Dominant forearm (left right) record:............					
Non-dominant forearm record:............					

135

B. Summary and discussion of data

a) What is the EMG electrode for? What does it measure?

....

....

....

....

....

....

b) Which of the examined forearms has higher strength capacity?

Subject 1: dominant non dominant no difference

Subject 2: dominant non dominant no difference

Is there any difference between the subjects? Explain the observation!

....

....

....

....

....

....

c) Is there any difference between the subjects' maximal strength capacity?

yes no

Explain your observations!

....

....

....

....

....

....

d) Calculate muscle strength per EMG unit during approximately same strength of clench! (Divide maximum muscle strength (in kg) by the corresponding integrated EMG amplitude (in millivolts)!)

1. subject dominant:...

1. subject non dominant:...

2. subject dominant:...

2. subject non dominant:...

Explain and interpret the order above!

....

....

....

....

....

e) Compare the fatigue length of the forearms! (1: shortest; 4: longest)

__ 1. subject dominant __ 1. subject non dominant __ 2. subject dominant __ 2. subject non dominant

Explain the result!

....

....

....

....

....

f) When holding an object, does the number of motor units remain the same? Are the same motor units used for the duration of holding the object?

....

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....

....

g) As you fatigue, the force exerted by your muscles decreases. What physiological processes explain the decline in strength?

....

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....

C. Definitions

Motor unit...

....

....

Muscle tone:..

....

....

Motor unit recruitment:...

....

....

Fatigue:...

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Dynamometry:..

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6. data report and evaluation: Human perception - testing human vision, hearing, tactile and thermal sensation

Experiment was accomplished by

....

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Teacher:

....

Date:

....

Aims of the practical lesson *(brief definition in a few sentences)*

Introduction *(can be continued on a separate sheet if needed)*

1. Brief introduction to human senses – main characteristics of physiological features influencing visual, auditory, thermal or tactile perception *(summary of the most important facts in regard to the analysis of the practical data)*

Human vision

1. Structure of the human eye:

a) Opthalmoscopy on an artificial eye model

Summary of the observations:

a) Create a schematic drawing in the outlined area below indicating the most characteristic features seen inside the artificial eye model! Mark the position of the **blind spot (optic disc)** and the ***fovea centralis***!
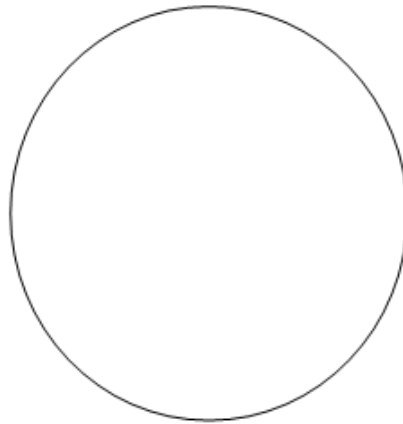
Choose 2 colours and shade that area where **cones** (................. colour) and ***rods*** (.................... colour) are located!

b) Which eye does the the artificial eye model represent?

left eye right eye

c) Is there a difference in the distribution of rods and conese? yes no

Does it influence any physiological properties of human vision?

....

....

....

b)use of the sclera lamp: blood vessel shadow experiment

a) Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

....

....

b) Summary of the observations:

....

....

c) What kind of information can be drawn from this experiment? How can you interpret the observed phenomenon?

....

2. Determining the field of view: perimetry

Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

....

....

....

....

Summary of the observations: number of the attached recording(s):................

which coloured field of view was tested? red green blue

a) Is there a difference between the size of the black-and-white or coloured field of vision?

yes no

Explain your findings!

....

....

....

b) Is there a difference between the position of the blind spot in case of the black-and-white or coloured field of vision? yes no

Where is the blind spot on the recordings?

....

Why does blind spot develop?

....

....

....

c) Is there a difference between the size of the normal (control) and the Subject's field of vision?

yes no

How can you explain it? How the size of the field of view is influenced?

....

....

....

....

....

3. Testing the sharpness of vision (Snellen tables)

Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

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....

....

Summary of the observations:

a) Does the Subject have normal vision (emmetropic eye)? yes no

If not, what kind of refraction abnormalities does (s)he have?

....

....

....

b) The figure below shows the cross section of a normal (emmetropic) eye:
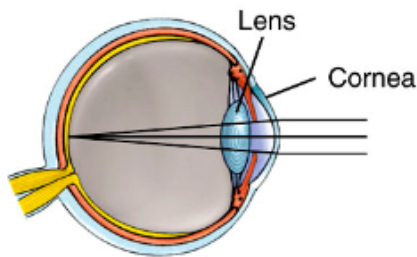
Where is the image focused?

....

c) Indicate on the left figure below where the image is focused in the Subject's investigated eye! In case (s)he needs any correction (e.g. in case of *myopia* (*nearsightedness*) or *hypermetropia* (*farsightedness*)), indicate on the right figure how the necessary correction can be carried out by glasses.

Subject's vision: myopia hypermetropia
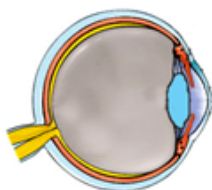
Subject's vision
(without any corrections)

how
can be
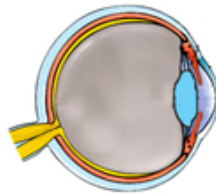
corrected vision

optical
correction
achieved?:

....

4. Testing spherical curvature problems: astigmatism

Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

....

....

....

....

Summary of the observations:

a) What kind of refraction abnormality is called as astigmatism?

....

....

....

b) Why is the Placido's disc useful to determine astigmatic eye?

....

....

....

c) How can astigmatism be corrected?

....

....

....

5. Testing colour blindness (Stilling-Ishihara tables)

Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

....

....

....

....

Summary of the observations:

a) When do we talk about colour blindness?

....

....

....

b) What type(s) of colour blindness can develop?

....

....

....

....

....

....

....

c) Who has the higher chance to have colour blindness? females males equal

How can you explain it?

....

....

....

....

....

Human hearing

1. Audiometry

Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

....

....

....

Summary of the observations: number of the attached recording(s):................

a) In which frequency range did the Subject have the lowest auditory threshold?

right ear: .... left ear:..

Was it the same as expected? yes no

Expain the observed findings and any differences between the observed and expected auditory threshold values!

....

....

....

c) Are there any diferences between the auditory values of the left and the right ear?

yes no

Explain the observed findings!

....

....

....

d) What kind of effects can lead to prominent increase in auditory threshold values? Give some examples!

....

....

....

....

e) How the pitch of the tone is influenced? How can we distinguish between higher and lower pitch?

....

....

....

2. Rinne's test: comparing bone and air conductance

Give a short description how the task was carried out (in case it is needed, continue on a separate sheet):

....

....

....

....

....

Summary of the observations:

a) In which case did you hear for a longer time the sound of the vibrating soundfork?

in case the vibrating soundfork was placed

to the top of the head .... to the teeth

to the teeth .... close to the ear

to the top of the head .... close to the ear

b) In case of the Subject,

air conductance bone conductance was more efficient

c) Explain your findings! Did you get the expected changes? If not, what can be the reason for the differences?

....

....

....

....

....

d) How and when can it happen that bone conductance works more efficiently than air conductance?

....

....

....

....

Tactile and thermal sensation

1. Investigating thermal sensations

a) adaptation of thermoreceptors

Summary of the observations:

a) When the two hands were immersed at the same time to cold or warm water, in which case did you observe faster adaptation

cold receptors warm receptors

b) Explain your observations!

....

....

....

....

....

....

b)relative sensitivity of thermoreceptors

Summary of the observations:

a) What did you observe when the two hands, adapted previously to cold or warm water, were immsersed at the same time to room temperature water?

in case of the hand immersed previously to warm water: ....

....

in case of the hand immersed previously to cold water: ....

....

b) Explain your findings!

....

....

....

....

....

2. Tactile sensitivity: testing mechanical discrimination thresholds

Summary of the observations:

a) List the discrimination threshold values determined in the following areas:

fingerpad:...................... palm:................................ back of the hand: ....

lower arm:.............................. upper arm:................................ back:.............................

tight:.................................

b) Explain your observations!

....

....

....

....

....

....

Definitions

sensory threshold:..

....

sensory discrimination:..

....

decibel:...

....

dioptria:...

....

perimetry...

7. data report and evaluation: Electroencephal- ography (EEG) – examination of changes in the beta- and alpha activity

Experiment was accomplished by:

....

....

....

....

Teacher:

....

Date:

....

Subject's profile

Name: .... Height: ....

Age: .... Weight: ....

Gender: male / female

The aim of the experiment *(brief definition in a few sentences)*

Introduction *(can be continued on a separate sheet if needed)*

1. Mechanisms related to the generation of EEG, basics of the EEG recording, typical EEG waveforms (summary of the most important facts in regard to the analysis of the practical data)

2. Methods and materials (schematic enumeration)

3. Technical details of the practical exercises (preparation of the subject, settings of the recording system, explanation of the exercises)

A. Data and Calculations

After opening of the Lesson04 (EEGII) recording, the following signals can be seen on the display: **channel 1(CH1) – recorded („raw") EEG signal; channel 40 (CH40) – alpha activity** (after digital filtering of the EEG signal)**; channel 41 (CH41) – alpha rms.** Alpha rms is the root mean square value of the signal within a window width of 0.25 seconds. This parameter provides a good characterization for the actual quantity of the alpha waves.

Attach the printouts from the magnified, analyzed recording segments to the lab record and note the number of the attachments.

1. Determination of the amplitude as well as frequency of alpha waves

Name a segment from the EEG recording

when you expect high **alpha** activity: ....

Number of the segment: ....

Analyze the signal seen on **channel 1 (**recorded „raw" EEG signal). Magnify the selected section by the **Zoom** cursor then set the amplitude of the signal by the **Display/Autoscale Waveforms** function**.** Use the following measurement windows: **channel 1 (raw EEG) – p-p** (peak-to-peak; this measurement shows the difference between the maximum and minimum amplitude value in the selected area); **channel 1 (raw EEG) – delta T.** Identify the consecutive alpha waves. Select an alpha wave by the I-beam cursor <u>from the beginning of the wave to the peak of the wave.</u> By this kind of selection, you can measure the amplitude of the wave. Then insert the value seen in the **channel 1 (raw EEG) p-p** measurement window to the p-p (amplitude) line of the table below. After that, select the same alpha wave <u>from the beginning to the end</u>. Period time of the wave will apear in the **channel 1 (raw EEG) – delta T** measurement window. Insert this value to the table below. (Delta time can be negative if the selection had been made in a reverse order i.e. the end of the wave was selected first and the wave onset last). Repeat the above mentioned procedure to 7 different, consecutive alpha waves. Calculate frequency values from the period time values. Calculate the mean of the amplitude-, period- as well as frequency values.

.	EEG waves (alpha activity)								Mean
	1.	2.	3.	4.	5.	6.	7.	8.	
p-p (amplitude) [μV]									
delta T (period time) [s]									
frequency [Hz]									

Number of the attachment:..................

2. Determination of the amplitude as well as frequency of beta waves

Name a segment from the EEG recording

when you expect high **beta** activity: ....

Number of the segment: ....

Analyze the signal seen on **channel 1 (**recorded EEG signal). Analysis should be made in a similar way as in Exercise 1.

Number of the attachment:..................

	EEG waves (alpha activity)								Mean
.	1.	2.	3.	4.	5.	6.	7.	8.	
p-p (amplitude) [µV]									
delta T (period time) [s]									
frequency [Hz]									

3. Effects of different interventions on the quantity of alpha waves

Analyze the signal seen on **channel 41 (alpha rms).** Magnify the selected section by the **Zoom** cursor then set the amplitude of the signal by the **Display/Autoscale Waveforms** function**.** Use the following measurement window: **channel 41 – mean.** Take notice of the recorded EEG (channel 1) and select a 2-seconds-long part from every different recording segments (1st, 2nd, 3rd). Read the alpha rms values appearing on the measurement window and fill them into the table below**.**

When you select a part from the 1st (control) segment, pay attention to select a segment where several alpha waves can be seen in the recorded EEG (channel 1). In case of the 2nd segment (mental math), select that segment for the analysis which shows the data recorded immediately after the order to calculate (when the subject likely shows maximal mental activity). Take care to select artifact-free epochs. Alpha rms value of the first segment is the reference (control). Calculate alpha rms difference between control and experimental stages (i.e. 2nd and 3rd segments).

	mean alpha rms (Ch 41 - mean) [µV]	alpha rms difference compared to the control (experimental - control) [µV]	evaluation (increase / decrease / no change)	number of the attachment
control: eyes closed, quiet state (1st segment)				
mental math (2nd segment)				
eyes open (3rd segment)				

4. Artifacts

Does the recording contain artifacts? Yes No

If artifacts are present in the recording, mark them on the printouts and reference them by numbers. Identify the types of the artifacts.

Number of the attachment:..................

....

....

....

....

B. Data summary and conclusions

1. Under which intervention did you observe the largest amount of alpha waves? Use the table at Exercise 3.

....

....

2. Under which intervention did you observe the smallest quantity of alpha waves? Use the table at Exercise 3.

....

....

3. How did the quantity of alpha waves change by the degree of attention?

....

....

....

....

....

4. Based on the experimental results, did the subject pay attention to the mental math task?

yes no

5. Compare the EEG patterns recorded with eyes open or closed.

a) What kind of changes did you observe in the EEG when the subject's eyes were open?

....

....

....

....

....

b) What happens to the functioning of the occipital lobe neurons when the subject's eyes are open?

....

c) Give a definition describing the changes in the functioning of the the occipital lobe neurons when the subject's eyes are closed!

....

d) When did you observe more pronounced beta activity?

eyes closed eyes open

C. Analysis of a healthy and a pathological EEG recordings

Compare the EEG recorded from a healthy subject (26 years old male with regular cortical activity) with the EEG originated from a **subject with unknown medical condition** (thus, he/she can be healthy, might suffer from some illnesses, can be a child or adult, etc). EEG was recorded using the international 10-20 system. Electrode configuration can be seen on Figure 1.
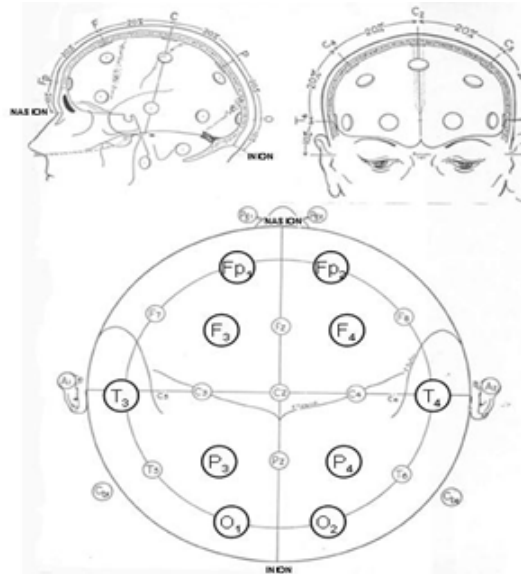
Electrode configuration in the international 10-20 system. Fp: frontopolar; F: frontal; P: parietal; O: occipital; T: temporal.

Use calibration bars indicated on the recordings to determine the **amplitude** as well as the **frequency** of typical EEG waves. For the calculation, mark the start and end points of the analyzed segment on the recording (for example, an alpha spindle). Count the waves then measure the length of the analyzed segment. Calculate the duration of the segment in proportion to the time calibration bar. Then divide the number of the waves by the duration of the analysed segment to obtain the frequency of the given waves. [For example: time calibration indicates 25 mm as 1 second. The length of the selected epoch is 23 mm. Thus, the duration of the epoch is 0.92 s. If there were 10 waves in the selection, then the frequency is 10/0.92=10.87 Hz.]

There is no need to provide exact measurement on the frequency and amplitude of beta activity. Instead, indicate directly on the EEG recordings and also, in the table below the lead(s) where beta activity is clearly visible.

If artifacts are present in the recording, mark them on the printed recording and reference them by numbers. Identify the types of the artifacts.

The healthy subject was in a supine position with closed eyes during the recording. Note that the subject with unknown medical condition is not certainly diseased.

Compare the EEG of the healthy subject with the subject with unknown medical condition.Give a detailed reasoning in the lab report about your opinion! It is not the medical accuracy of the diagnosis but the precision and depth of the analysis what will determine the rating of the lab record.

Characterization of typical EEG waveforms	Recording of the healthy subject			Recording of the subject with unknown medical condition; number:............		
leads	amplitude [µV]	frequency [Hz]	name of the waveform	amplitude [µV]	frequency [Hz]	name of the waveform
Fp$_1$ - F$_3$						
F$_3$ - T$_3$						
T$_3$ - P$_3$						
P$_3$ - O$_1$						
Fp$_2$ - F$_4$						
F$_4$ - T$_4$						
T$_4$ - P$_4$						
P$_4$ - O$_2$						
T$_3$ - Fp$_1$						
Fp$_1$ - Fp$_2$						
Fp$_2$ - T$_4$						
T$_4$ - O$_2$						
O$_2$ - O$_1$						
O$_1$ - T$_3$						

1. Identification of disordered waveforms on the recording of the subject with unknown medical condition:

a) name the type of the disordered waveforms and report the leads where those appeared.

....

....

....

b) What was the cause of the appearance of the disordered waveforms?

....

....

....

2. Identification of artifacts:

a) Did you observe artifacts in the recordings? If yes, on which leads?

recording of the healthy subject: ....

....

recording of the subject with unknown medical condition:...

....

b) What was the possible cause of these artifacts?

....

....

....

3. Analysis of the recording belonging to a subject with unknown medical condition:

a) What do you think about the experimental conditions? Was the patient asleep or awake during the recording?Were the subject's eyes closed or open during the recording? Explain your answers!

....

....

....

....

....

....

....

b) What is your opinion about the age of the unknown subject? Explain your answer.

....

....

....

....

....

c) What is your opinion about the health status of the unknown subject? Explain your answer.

....

....

....

....

8. data report and evaluation: Studying animal behavior - examination of animal learning and behaviour

Experiment was accomplished by

....

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Teacher:

....

Date:

....

**Goals of the practical lesson** _(brief definition in few sentences)_

**Introduction** _(can be continued on a separate sheet if needed)_

**1. Short explanation on the possibilities to observe animal learning and behavior** _(summary of the most important facts in regard to the analysis of the practical data)_

**2. Methods and materials** _(schematic enumeration)_

1. _**Examination of animal behaviour**_

Explain the course of the experiments shortly at every exercise and explain your results. Attach the activity diagrams showing the different behavioral patterns to the lab record.

1. Open field test: behaviour of a single rat during quiet conditions

Short description of the experiment (Give the outline of the open field chamber, e.g. the orientation and marking of the observation field):
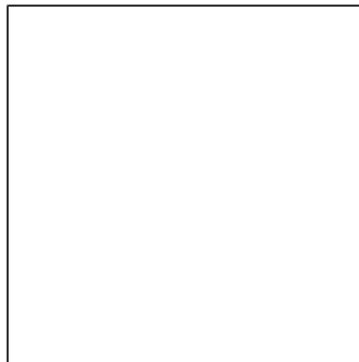
Make a list of the recorded behavioral items:

....

....

....

Describe the route of the observed rat which was accompanished during the observation period (5 minutes):

....

....

....

Number of the attached activity diagram:................................

Summary of the observations*(can be continued on a separate sheet if needed)* (What kind of typical behavioral items did you observe immediately after placing the animal into the open filed? Was the animal's behaviour different during the initial observations or after several minutes? Did you observe any stereotypic behavioral patterns? What were the regions of the open field which were preferred by the rat? Which of the recorded behavioral items reflected especially the exploratory activity of the animal?)

....

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2. Open field test: behavior of a single rat in case of disturbing („frightening") conditions

Short description of the experiment:

....

....

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....

....

....

Describe the route of the observed rat which was accompanished during the observation period (5 minutes)!

....

....

....

Number of the attached activity diagram:................................

Summary of the observations*(can be continued on a separate sheet if needed)***:**

(What kind of typical behavioral items did you observe immediately after the application of the „frightening" stimulus? Was the animal's behaviour different during the initial observations or after several minutes? What were the regions of the open field chamber preferred by the animal? How frequent was the exploration? How did the behavioral patterns differ before and after the application of the „frightening" stimulus?)

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3. Open field test: testing behavioral differences between an adapted and a newcomer rat within the same open field

Short description of the experiment:

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Describe the route of the observed rat which was accompanished during the observation period (5 minutes)!

1. "adapted" animal:

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2. "newcomer" animal:

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Number of the attached activity diagram:

1. ("original") animal: .... **2. ("new") animal:** ....

Summary of the observations*(can be continued on a separate sheet if needed)***:**

(Which were the typical behavioral items showed by the animals? Which regions of the open field were preferred by the animals? How frequent was the exploration? Did any signs of social interaction appear between the animals? How did the behavior of the „adapted" animal change in response to the placement of the „new" animal? How the

behavior of the „new" animal was similar to the behavior of the „old" animal when the animal was originally placed to the open field chamber (see exercise 1)?)

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B. Examination of animal learning

1. Operant conditioning:training of a thirsty rat in the Skinner-box

Short explanation of the experiment:

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Pronounced stages during the training procedure (*can be expanded if needed*)**:**

Name of the pronounced behavioral stage	Time elapsed since the start of the experiment (approximately, min)

Summary of the observations *(can be continued on a separate sheet if needed)***:**

(What kind of typical behavioral did you observe after placing the animal into the Skinner-box? Did you experience any stereotyped behavioral patterns? Was the animal motivated to get some sweet water? What was the strategy of the experimenter during the training procedure? Did the animal learn to press the response lever?)

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2. Examination of spatial learning: training of a thirsty rat in the Y-maze

Short explanation of the experiment:

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Selected directions during the consecutive trials (*can be expanded if needed*)**:**

Direction selected by the experimenter: right left

Number of the trial	Direction selected by the animal		Number of the trial	Direction selected by the animal	
	right	left		right	left
1.			11.		
2.			12.		
3.			13.		
4.			14.		
5.			15.		
6.			16.		
7.			17.		
8.			18.		
9.			19.		
10.			20.		

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Summary of the observations*(can be continued on a separate sheet if needed)***:**

What kind of typical behavioral items were showed by the rat inside the Y-maze? Was the animal motivated to search for the sweet water? What was the strategy of the experimenter during the training procedure? How much time did the animal need to learn the right direction to the water?

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C. Definitions

Operant conditioning: ....

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Thorndike's law of effect: ....

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unconditioned reflex: ....

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conditioned reflex: ....

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9. data report and evaluation: Investigating drug-mediated effects on animal behaviour - Irwin's activity test

Experiment was accomplished by

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Teacher:

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Date:

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<u>***Goals of the practical lesson***</u> *(brief definition in few sentences)*

<u>***Introduction***</u> *(can be continued on a separate sheet if needed)*

1. Short explanation of the importance of testing animal behavior during the development of human medicines *(summary of the most important facts in regard to the analysis of the practical data)*

2. Methods and materials *(schematic enumeration)*

1. ***Description of the observed behaviour elements***

Fill out the following table according to your observations! The behaviour and certain reactions of the animals are recorded and scored between 0-8 points. During the scoring, the following pattern is followed: when an activity/characteristic is also present in the control animals (like exploration, body posture or normal skin colour), then control, non-treated mice are given 4 points. In this case, animals receiving the test compound will have higher or lower scores when their activity is increased or decreased in comparison to the control mice' activity, respectively. In those cases when control mice do not normally show the investigated effects (like urination, salivation, tremor, etc), then control mice receive 0 point and the tested mice will be given increasing amount of points according to the severity of the symptoms.

	normal value (control)	mark	mark	mark
species: sex: date of examination:..				
1. urination	0			
2. salivation	0			
3. exploration	4			
4. alertness	4			
5. self-cleaning	4			
6. sponteneous activity	4			
7. restlessness	0			
8. stereotypic behaviour	0			
9. vocalisation	0			
10. colour of the skin	4			
11. piloerection (fuzzy hair)	0			
12. torment	0			
13. tremor	0			
14. spasms	0			

	normal value (control)	mark	mark	mark
15. trepidity	0			
16. body posture	4			
17. tension and position of the limbs	4			
18. abnormal walking, ataxia	0			
19. irritability	0			
20. wincing (towards loud noise)	0			
21. pain rection (towards pinching)	4			
22. cornea reflex	4			
23. external ear reflex	4			
24. flexor reflex	4			
25. closure of the eyelids	4			
26. goggling eyes	0			
27. fear reaction	0			
28. reactivity (on the table)	4			
29. placing effect	4			
30. passivity (lack of escaping)	0			
31. strength of gripping	4			
32. body tension	4			
33. tension of the limbs	4			
34. aslope plate (absence of motor coordination)	4 (also in degree)			

Create a graph from the recorded scores, using different colour or label for the differently treated animals!
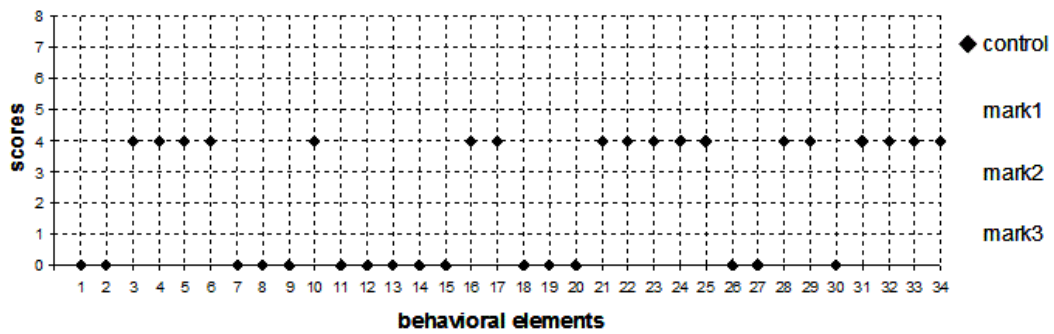
1. *Evaluation and interpretation of the observations*

1. Which behavioural elements were altered to the highest extent in case of the different treatments? Fill out the table with the number(s) of the corresponding behavioral elements!

marking of the animals	in comparison to the control values, treated animals received	
	increased scores	*decreased scores*

2. Summarize the most prominent behavioural changes observed in case of the different treatments!

marking of the animals	characteristic behavior during the observations
control mouse	

3. According to your observations, determine which type of treatment did the treated animals receive!

marking of the animals	type of treatment

Give a detailed reasoning how your final assumptions were made:

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C. Definitions

Irwin's activity test: ....

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motor coordination: ....

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body tone: ....

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behavioural effects: ....

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10. data report and evaluation: Polygraphy: Galvanic skin response, ECG and respiration measurements, - „lie detection"

Experiment was accomplished by

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Teacher:

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Date:

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Subject's profile

name: height:

age: weight:

gender: male / female

__Aims of the practical lesson__ (brief definition in a few sentences)

__Introduction__ (can be continued on a separate sheet if needed)

__1. Physiological background of polygraph recordings – main characteristics of physiological features changing under excitement state__ (summary of the most important facts in regard to the analysis of the practical data)

__2. Methods and materials__ (schematic enumeration)

__3. Technical details of the practical exercises__ (preparation of the subject, settings of the recording system, explanation of the exercises)

A. Data and calculations

I. Setting up the measurement

Mark the appropriate cells of the table accordingly to the changes of the galvanic skin response (CH3) observed in the following tasks! **Attach the analyzed segments as printouts to the lab report!**

task	Galvanic skin response [ch3]		
	increase	*decrease*	*no change*
Quiet			
Saying his/her own name			
Countdown from 10			
Countdown with increasing odd numbers from 30			
Touching the face			

Did you observe any significant changes in the respiratory rate or in the hearth rate? Why?

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Number of the attachment:.....................

II. Effects of words with various emotional charge

Mark the appropriate cells of the table accordingly to the changes of the galvanic skin response (CH3) observed after the pronounced words! **Attach the analyzed records as printouts to the lab report and indicate the appropriate words above the responses!**

words	Galvanic skin response [ch3]		
	increase	decrease	no change

Number of attachment:.....................

III. Lie detection

1. Lists the asked questions, and mark the answers (Y: yes; N: no) of the examined person. Mark in the table how the measured values changed to the baseline after the questions (increased/decreased/did not change)! Based on the results, indicate your conclusion whether the examined person told the truth (T: truth) or lied (UT: Un-truth)! Attach the analyzed sections as printouts - don't forget to indicate the number of the corresponding question above the recording!

question	answer	Galvanic skin response [ch3]			Conclu-sion
		increase	*decrease*	*no change*	
1.	Y N				T UT
2.	Y N				T UT
3.	Y. N				T UT
4.	Y N				T UT
5.	Y N				T UT
6.	Y N				T UT
7.	Y N				T UT
8.	Y N				T UT
9.	Y N				T UT
10.	Y N				T UT

When did you observe significant changes in the rhythm of the breathing or in the heart frequency?

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Number of the attachment:....................

1. List the numbers in the order of asking and mark the answers (Y: yes; N: no) of the examined person. Mark in the table how the measured values changed to the baseline after the questions (increased/decreased/did not change)! Based on the results, indicate your conclusion whether the examined person told the truth (T: truth) or lied (UT: Untruth)! What was the drawn number? Attach the analyzed sections as printouts- don't forget to indicate the number of the corresponding question above the recording!

asked numbers	answer	Galvanic skin response [ch3]			conclusion
		increase	*decrease*	*no change*	
	Y N				T UT
	Y N				T UT
	Y. N				T UT
	Y N				T UT
	Y N				T UT
	Y N				T UT
	Y N				T UT
	Y N				T UT
	Y N				T UT
	Y N				T UT

When did you observe significant changes in the rhythm of the breathing or in the heart frequency?

……………………………………………………………………………………………

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B. Data summary and conclusions

a) Why is the setting of the measurement (environment, circumstances, participants, recording basic activity, etc) an important element during polygraph investigations?

.………

b) When you set up the measurement, which task(s) evoked significant changes in the galvanic skin response values? Explain your experiences!

.………

c) Did you observe any difference between the measured and the expected effects of words with various emotional charges? Can you explain your experiences?

....

d) How can you interpret the recorded effects of words with various emotional charges? How can these reactions be useful during psychological analyses?

....

e) Can you establish the wrong answer(s) among the correct ones? What was the principle of your conclusion?

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g) What major physiological changes account for the galvanic skin response?

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h) Give three reasons why polygraph testing of a person's sincerity and honesty may yield inconclusive results!

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