Medical Faculty
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Practical lessons in Pathophysiology

Selected chapters

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EXPERIMENTAL GASTRIC ULCER IN THE RATS

(R. Beňačka)

Theoretical background

Gastric and duodenal ulcer are the commonest diseases associated with hypersecretion of gastric juice basally, after exogenous stimulation, or following food. In line with well known old adage " No acid, no ulcer", both previous and recent studies showed repeatedly, that the major factor involved in pathogenesis of ulcer disease is increased level of hydrohlorid acid. This is released normally from the parietal cells of oxyntic mucosa in gastric body and fundus into the lumen in response to various stimuli.

The major stimulus is neurogenous efferent input from vagus nerve, acting both during so called cephalic phase of gastric secretion or later during eating by local vago-vagal reflexes. It is known that postganglionic vagal efferents synapse directly with gland cells using an acetylcholine as an active agent at the neuroeffector cell junction. The final mediator , or possibly comodulator of this a cholinergically-induced release of gastric acid is histamin, released from mast cells scattered throughout the gastric fundus or the body upon a neural-vagal stimulation or by direct distension of gastric wall. These physiological data appeared to be important not just in pathogenetical considerations, but also from the therapeutical point of view, since *vagotomy, anticholinergics* or *antihistamine drugs* , especially *H_2-class of receptor blockers)* appears at present to be most effective in treatment of disease.

Large amount of acid is also released during so called gastric phase of secretion in response to the presence of food, alkali, calcium and fluids in the stomach, as well as to mechanic distension. Is now apparent that the most powerful stimulants of gastric acid secretions in consumed food are proteins, particularly small peptides or aminoacids. They were shown to increase gastric acid concentration indirectly by acting on antral-pyloric G cells, which secrete gastrin. This hormone, acting both in paracrine manner or through blood circulation, stimulates parietal cells as well as histaminergic and peptinergic cells in the gastric body and fundus thus enhancing both acid as well as pepsine content oi gastric juice. The above mentioned role of a proteins in triggering acid-pepsin secretion is may provide a likely explanation of higher incidence of ulcer disease in industrial countries, since high intake of protein-enriched food is a common *dietary customs* in many of these communities. Accompaying stress may act as additional factor.

Increased level of proteolytic enzyme pepsin in gastric juice is for a longer time recognized to be another important factor involved in pathogenesis of gastric ulcer disease. Pepsin is formed normally in acid enviroment from pepsinogen, which is released from chief cells in oxyntic gland mucosa of the gastric fundus and the body after distension of stomach or vagal stimulation. It is known from practise, that if less concentrated acid, pepsin is a factor, which is definitely required to produce the ulcers in many cases. Importantly, some recent data suggest, that altered composition or abnormal release of pepsinogen I , may be the major defect shown in some autosomal dominant *genetically induced* ulcer diseases .

In spite that the "acid-pepsin" theory of ulcer disease has been for longer time advocated, it seems that none of above factors can be alone the cause of ulcer, except possibly only a excessive hypersecretion increased by tumor overproduction, as in gastrinoma (Zollinger - Ellison syndrome, nonbeta islet tumors of the pancres). Recently, it was shown that the defective local tissue resistance may be as critical factor in pathogenesis of ulcers as acid or pepsin hyperproduction. This may imply impaired mucosal blod flow, composition of secreted mucous glycoproteins ands mucopolysaccharides or perturbed functional mucosal barrier (tight junction between the collumnar epithelial cells). The circumscription of the lesions to a relatively small area of the gastric mucosal surface bathed by acid-peptic juice supports this presumption. It is likely that impaired local tissue resistance is the common pathogenetic denominator of many other ethiological factors involved, e.g. heavy smoking, abusing of alcohol or chronic stress, etc. Decreased mucosal resistance, perhaps mediated by altered glycoproteins in mucus barrier was shown to be also important in ulcers associated with ABO bood groups, or other genetically or hereditary based disorders as shown in many epidemiologic studies.

Experimental protocol

Method I

Two Wistar rats starveling 24 hours are anaesthetized with intraperitoneal chloralose (400mg pre kg) and suspended prone on operation table with limbs affixed cross. After the abdominal skin has been shaved and dizinfected by flavin, wide medial longitudinal laparotomy is performed by cutting the skin, muscles and peritoneum in a usual manner. Stomach is gently freed from peritoneum by blunt probe and its antral-pyloric segment close in to duodenum, is ligated by silon thread. Abdominal wall is sawn in two layers (muscles and

skin), the skin is covered by dizinfective powder and aninal is left to recover from surgery in well pre-heated box. Approximatelly 24 hour later the animals are reanaesthetized, the abdominal cavity is reopened and the effect of pyloric ligature in gastric mucosa is observed. The gastric wall is cut along the large curvature from just above the ligature up to gastric cardia, gastric cavity is opened and the mucosal surface is washed out by saline. Gastric mucosa in rats differs in its structure from the human one. It consists typically of two clearly separated regions: yellowish coloured membranaceous part, situated rostrally and pink-brown coloured glandular oxyntic mucose occupying more caudal parts of the gastric body and fundus. Peptic ulcer lesions can be observed in the both parts of mucosa, although mostly they are limited to glandular mucosal surface. The lesions are typically of pinhead- or mill size, and rounded, oval, or logitudinal in shape. Haemorrhage spots can be found inside mostly. Likely explanation for such a experimantal peptic ulcer induced by pyloric ligature is a long duration stagnation of acid - pepsin content of gastric juice in the lumen. Additional factors, like a lower resistance of mucosal membrane or disturbed blood supply in the mucosa of fundus caused by a ligature may apply, as well. Traumatic impairment of stomach during preparation as well as stress-induced circulatory and secretory alterations in gastric mucose, however, can not be principally ruled out.

Method II

In addition to mechanical insults, gastric peptid ulcer can also produced experimentally by an application of various chemicals, e.g. histamin, epinephrin, vasopresin, or caffein. In present experiment gastric ulcer will be produced by phenylbutasone, which produce toxic lesions in gastric mucose, and accompanyingly it enhances gastric acid release by acting locally on parietal gland cells. The drug is administered intraperitoneally in two 24-hour starveling rats in dose 150 mg per 100 mg b.w.. The peptic ulcer lesions produced by drug in the gastric mucosa are observed 24 hour after it has been applied using similar preparation

Evaluation

1. Describe common features or differences between the gastric ulcer lesions in humans and experimental animals. 2. Explain the possible causes and mechanisms of peptic ulcer disease in human patients and suggest another possible way how these factors carred be modelled experimentally.

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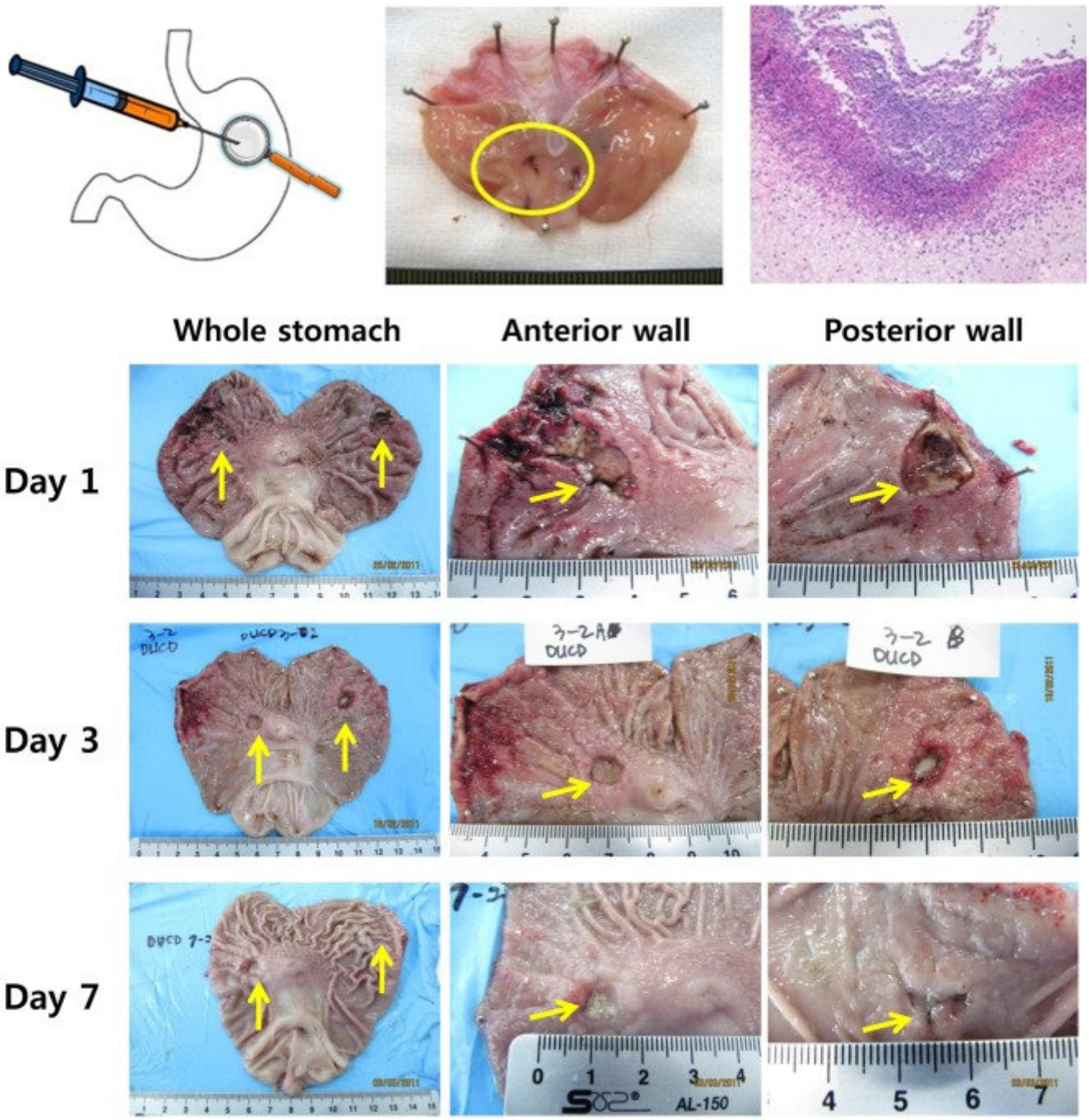

Fig.1. Rabbit experimental model of acetic acid-induced gastric ulcer. Ulcer margins and area are generally undetectable without microscopic observation. (A) Rat gastric ulcer induced by intra-luminal injection of acetic acid and its macroscopic and microscopic observation. Ulcer area is controlled by ring forceps. (B). After injection of acetic acid erosions or ulceration can be observed on mucosa (as verified by histology) accesible for periodic checkout thorugh permanent gastrostomy (opening into the stomach exposed throgh the skin).

Adapted from: Maeng JH1, Lee E, Lee DH, Yang SG.: Rabbit gastric ulcer models: comparison and evaluation of acetic acid-induced ulcer and mucosectomy-induced ulcer.Lab Anim Res. 2013 Jun;29(2):96-102. doi: 10.5625/lar.2013.29.2.96. https://www.researchgate.net/publication/245029800_Rabbit_gastric_ulcer_models_comparison_and_evaluation_of_acetic_acid-induced_ulcer_and_mucosectomy-induced_ulcer

AUDIOGENOUS EPILEPTIFORM SEIZURES IN RATS

(R. Beňačka)

Theoretical background

Definition. Epilepsy is a disorder of brain function caused by abnormal synchronous and excessive activation of large neuronal ensembles characterised by recurrence of short-term paroxysmal episodes of disturbed consciousness, pathological electrical brain activity (EEG) and altered performance of motor and other cognitive, sensory and vegetative functions called *epileptic seizure*. Typical for epilepsy is repetitive occurrence of seizures throughout relatively long life period; sporadic fits, even if they display similar symptomatology *(epiletiform)*, can not be still considered as an ongoing epileptic disorder. Most commonly, such a occasional seisure might reflect momentary discordance in constellation of various outer and inner factors influencing brain activity (e.g. metabolism, oxygen supply, glucose intake, circulation disorders, etc.).

Etiopathogenesis. The disorder itself was likely recognized prior to the development of the earliest civilisations and its present incidence is estimated to range from 0.5 to 2% of general population. It has multifunctional etiopathogenesis reflecting both *genetical* and various *acquired* factors. Former imply for various single gene disorders (e.g. inborn errors of metabolism) or other hereditary influences evolving during intrauterine stage. Little is known about them; most likely they become clinically manifested only after they interact with other exogenous factors during postnatal ontogenesis. In later case the occurrence of epileptic fits is more or less clearly linked with either recent or ongoing and/or latent or clinically manifested brain disorder, henceforth this type is called *symptomatic epilepsy.* The causes are heterogeneous, e.g. various infections and inflammations of the central nervous system (CNS), brain tumours, cerebral vascular lesions, congenital malformations, systemic or cerebral metabolic disturbances, transient or prolonged hypoxic or ischaemic attacks, endogenous and exogenous toxins, drugs, impairments like perinatal and postnatal cerebral trauma and many others, as summarised in tab.1. Many times a period of months or years can elapse between the impact of well-known endo- or exogenous noxa and the appearance of clinical symptoms of epilepsy, what call an examiner search detailly for an insult in the patient's family history, e.g. postraumatic epilepsy after cerebromeningeal concrescence or postinfectional epilepsy. For these and similar cases the term *residual epilepsy* has been adopted. In spite of the advent of modern diagnostic techniques, includ-

ing CTs, scintigraphic scans or electrophysiology, in many cases, we can not find an evidence of organic brain lesion or functional disturbance responsible for the fits, nor do these attacks usually have a stable and focal onset in particular portion of the body, that would help us to anticipate the regions of brain involved. These cases are called *idiopathic epilepsy.*

Whatever distinct the causes of epilepsy are, they have apparently the same consequence, identical mechanism - an extreme autogenerously amplified excitation of smaller or larger ensembles of neurones in one or several restricted areas of the brain which, after the excitation overflow certain limits, has a tendency to burst out and spread over the adjacent regions up to distant areas of the brain. The tendency towards the epileptic seizure is determined by the "convulsive threshold" that usually shows considerable variation from one person to another. Those with very high threshold would never suffer a convulsion, no matter how severe the exciting stimulus is, while those in whom it is low are often the "idiopathic" epileptics who have attacks without apparent cause. It is difficult to draw a clear line of demarcation between the epileptic and nonepileptic in those whose convulsive thresholds falls between these two extremes. Not more that 50% of patients with penetrating brain injuries develop post-traumatic epilepsy. The convulsive threshold is clearly constitutional and heredity factor is probably important. In addition to convulsive threshold, another important variable is the resistance of brain tissue to the spread of the epileptic discharge. This is the property that is largely independent of the convulsive threshold. If this resistance is low, there is greater possibility for abnomal locus to generalise the wave of excitation and spread it off, a focal lesion thus giving a general convulsion with immediate loss of consciousness and no more apparent localising features. If the resistance is high, whatever low the convulsive threshold is, the epileptic manifestations will remain localised to the appropriate area of the body (according to the somatotopic cortical area involved) and consciousness may be comparatively unimpaired during the attack. When the resistance is intermediate between these two extremes, the onset of "epi-fit" is local but will certainly develop into a generalised seizure with transient unconsciousness.

Manifestation of commoner types of seizures. (Tab.2). The **petit mal "absence"** is a transient interruption of consciousness lasting for only a few seconds, beginning almost invariably in childhood and accompanied sometimes by flickering of the eyelids, and less often by brief jerky movements of the hands. The attack may be often precipitated by

Tab. 1. Some important causes of symptomatic epilepsy

Local causes

a) *Focal intracranial lesions sometimes associated with increased intracranial pressure*
Intracranial tumor, cerebral abscess, subdural hematoma,angioma or hematoma

b) *Inflamatory and demyelinating conditions*
Meningitis, all forms of acute and subacute encephalitis, toxoplasmosis, neurosyphilis, multiple sclerosis, cerebral cysticercosis

c) *Trauma*
Perimatal brain injury and/or hemorrhage, head injuries of later life

d) *Congenital abnormalities*
Congenital diplegia, tuberous sclerosis, porencephaly

e) *Degenerations and inborn errors of metabolism*
Cerebral lipidoses, diffuse sclerosis and leukodystrophies, ecephalopathies of infancy and childhodd, Pick's disease, Alzheimer's disease, progressive myoclonic epilepsy, subacute spongioform encephalopathy, Creutzfeld-Jakob's disease

f)) *Vascular disorders*
Cerebral atheroma, intracranial hemorrhage, thrombosis, embolism, eclampsia, hypertensive encephalopathy, cerebral complications of connective tissue (collagen) disease, polycythemia, intracranial aneurysm, acute cerebral ischemia from any cause

General causes

a) *Exogenous poisons*
Alcohol, absinthe, cocaine, strychnine, lead, chloroform, ether, insulin, amphetamines, camphor, Metrazol, organophosphorus and organochlorine compounds used as insecticides,monoamine oxidase inhibitors, imipramine and its derivates, withdrawal of alcohol, barbiturates, and other drugs

b) *Anoxia*
Asphyxia, carbon dioxide poisoning, nitrous oxide anesthesia, profound anemia, ischemia

c) *Disordered metabolism*
Uremia, hepatic failure, hypoadrenalism, water intoxication, porphyria, hypoglycemia, hyperpyrexia, alkalosis, hyperkalemia, pyridoxin deficiency

d) *Endocrine disorders*
Parathyroid tetany, idiopathic hypoparathyroidism, pseudoparathyroidism

e) *Conditions associated particularly with childhood*
Rickets, acute infections ("febrile convolutions")

flickering light, or music and are recurring frequently throughout the day being characterised by typical wave-and-spike discharge in EEG. The seizure is likely caused by synchronous discharge arising in brainstem reticular formation, but mechanism of its formation and spreading is not understood.

A major epileptic fit, convulsion of **grand mal** type lead to abrupt loss of consciousness that may or may not be preceded by a warning or *aura.* There may be a momentary cry followed by a *tonic phase* of generalised muscular rigidity in which the tongue may be bitten. If standing, the patient fall to the ground and often injures himself. The tonic phase passes, usually within a few seconds, to be followed by a *clonic phase* of generalised jerking or convulsive movements in which there may be incontinence of urine or faeces. There is often then a variable period of *postepileptic confusion* or drowsiness, followed by a period of sleep, or else consciousness may be quickly restored. The general amnesia develops for the period of epileptic fit. Most if not all grand mal attacks are thought to arise locally in the cortex where the discharge activity from some supposed small focus is spread rapidly over the hemispheres. The prevalence of particular motor, sensory, vegetative or generously behavioural symptoms depend on from which functional area of cortex the discharges evolve and spread. Jacksonian attacks, which can sometimes be long-lasting without loss of consciousness, are one form of focal epilepsy, but so too are all

the complex variants of temporal lobe epilepsy. The attacks often show a progressive set of symptoms (such as the spread of paresthesias from tongue to face and hand to arm), reflecting the spread of anomalous discharge across contiguous areas of the cortex, or it may cross the corpus callosum to produce the mirror focus in the opposite hemisphere or else it spreads to the reticular substance initiating a corticothalamo-cortical discharge. During the grand mal EEG shows diffuse high-frequency waveforms.

Akinetic attacks are seizures in which consciousness is lost for short time and postural tone is temporarily inhibited so that the patient may suddenly fall down to the ground in spite of no consequent tonic or clonic motor accompaniments.

Tonic seizures are episodes in which the patient assumes an abnormal dystonic posture for a few seconds or minutes. Patient's limbs may be generally extended and rigid with back arched, resembling the decerebration rigidity or meningitis signs. Consciousness is usually retained in tonic seizures. This type of disorder likely implies the midbrain lesion due to, e.g. local compression and is of critical importance since it is seen sometimes in severe cases of cerebral palsy.

Myoclonus is a sudden involuntary shock-like contraction of one or several different groups of muscles that produces a rapid, jerky movement, usually involving one or more limbs. It represents a relatively often form of epilepsy, but it is necessary keep in mind that myoclonic jerks may also occur physiologically, e.g. during falling asleep. Similar sudden jerk or startle response occurs, too, in otherwise normal individuals as a consequence of overload with loud noise, music or any unexpected physical or emotional stimulus. Such irritable stimuli are more frequently a provoking factor for myoclonus seen in patients with basal ganglia's disorders involving red nucleus.

In *animals*, the epileptic attacks have usually even more polymorphous manifestations than in humans and sometimes they could be overlooked. Most commonly, it use to be characterised by a more or less developed sudden paroxysmal alteration in overall motor, vegetative and behavioural performance that appears to be in any way irrelevant to normal animal's behaviour. It may include paroxysms of muscle contraction, disoriented and uncoordinated running around the experimental chamber, high and long skipping, leaping, rotation around the vertical axis, or more passive behavioural signs as falling into the cataleptic stiffness, calm lying with a tonic spasm, extension of upper and lower extremities, etc.. Such an experimental epilepsy can be provoked by various stimuli like acute or con-

Tab.2. The clinical classification of epileptic seizures

Generalized seizures

Bilaterally symmetrical seizures without local onset

a) Absences (petit mal)
b) Bilateral myoclonus
c) Infantile spasms
d) Clonic seizures
e) Tonic seizures
f) Tonic-clonic seizures
g) Akinetic seizures

Partial seizures

Seizures beginning locally with:

a) Elementary symptomatology: motor, sensory, autonomic (Jacksonian epilepsy)
b) Complex symptomatology: impared consciousness, complex hallucinations, affective symptoms, automatism
c) Partial seizures becoming generalized tonic-clonic seizures

stant electrical stimulation, topical application of chemical convulsants to cerebral grey matter, systemic application of drugs, local ligature of arteries or freezing the brain. In "conditioned" animals the seizures could be evoked also by various strong sensory and sensitive inputs, which evoke an "alarm-stress response", e.g. photic or audiogenous stimuli, electrical shocks, etc..

Rats, and especially some disposed breeds, have very sensitive hearing apparatus and appear to be extremely sensitive to sound or noise in either infrasonic or ultrasonic frequency ranges. Such stimuli as thumbing of the bell, explosion dins, beating with metal to metal or grating a stone against a stone, clinking of glass, scraping or creaking, all these might provoke in rats convulsions resembling human's epilepsy and called owing to the cause as **audiogenous epileptiform (epilepsy-like) fits.**

Experimental procedure

Experimental audiogene epilepsy should be performed optimally in a sound-proof surrounding to strengthen the effect of the sound stimuli; if not available it may be carried out in an in otherwise quiet room with doors and windows closed. In our conditions, we shall try to provoke the epilepsy by ringing of the electromagnetic bell for about 1-3 min. The bell is mounted firmly in the round-shaped sound-proof experimental box covered by a transparent removable top through which we can easily observe the behaviour of animals inside. We take into the experiment 3-6 Wistar rats, divide them to three groups and mark with a different colour spot to make an identification easier. In first group, we perform a "conditioned" strengthening of the reaction to bell ringing by exposing the animals 2-3 days before the experiment to a 1-2 min bell sound. The simple tenet of this classical Pavlovian conditioning is to facilitate forming of the firm "neural bridge" between the particular stimulus and the natural adverse animal's reaction resulting finally in lowering the convulsion threshold and propelling the generalisation of epileptic discharge in brain tissue. The ani-

mals in the second group are given in the beginning of the experiment 1-1.25 ml of 10% caffeine per 100 g b.w. i.p. or strychnine in dose 0.5-1 mg per 100g b.w. i.p.. Both drugs act in CNS as so called *central analeptics* an thier effect implies generously a decrease of an excitability threshold of neurones. The rats in the third group serve as control.

Controls as well as those pre-conditioned and chemically pre-treated rats are placed together into the box and the bell is switched on. Behaviour of animals belonging to each particular group (recognised according to the coloured spot) is thoroughly noted. Usually, some few minutes after having been exposed to unpleasant stimulus, the animals show first marked changes in their behaviour, e.g. rapid uncoordinated runaway, chaotic tossing about the floor, rotating, high and long jumping or typical circling round the wall of the box resembling the run in the circus ring. With stimulus being prolonged to 3-5 min, the typical epileptiform motor symtoms use to develop, implying either tonic or clonic contractions or combined tonic-clonic spasms of different muscles. *Tonic spasms* are typicaly longer lasting contractions afflicting usually large muscle groups in limbs or affect sometimes musculature in the whole body. *Clonic spasms* are strong jerking movements of high frequency afflicting usually smaller muscles groups separately or in close succession. Sometimes they generalise and the head, trunk and limbs are rapidly flexed and extended in near synchronous manner. Otherwise, the spastic jerking may also be manifested by subtle tremorous movements.

In many cases, the initial phase of motor restlessness and agitation can be interrupted by a hypokinesia or akinesia. Animals are lying timid and calm in a corner of the chamber like in catalepsy, with body being like frozen stiff and limbs being hardly stretched even by stronger manipulation. In other cases we can move and shape the parts of animal's body like with the wax- figure, so called wax-stiffness (lat. flexibilitas cerea) often seen in parkinsonism. As the seizure subsides, the animals gradually recover and get aroused and oriented throughout several minutes. Sometimes, the epileptic attack may result in a total physical exhaustion of animals; incontinence of urine and faeces sometimes occur as a result of extreme stress.

The above mentioned symptoms can be prevented by application of 1 ml of 10% NaBr per 100g b.w. i.p. or half-normal anaesthetic dose of Na-pentobarbitone. Both these drugs, as well as e.g. diazepines, act generally as depressants of central nervous activity and highten the convulsive threshold, by this mean being antagonists to effects of strychnine or

caffeine. The latter drugs, on contrary, may be used in control animals, to enhance the precipitation of epileptic symptoms.

Evaluations

1. Note in your protocol the typical changes in rat's behaviour upon audiogene stimulus. 2. Compare the behaviour of control rats and those pre-conditioned and chemically pre-treated. 3. Evaluate, whether the epileptiform motor manifestations could be prevented by central depressants of neural activity.

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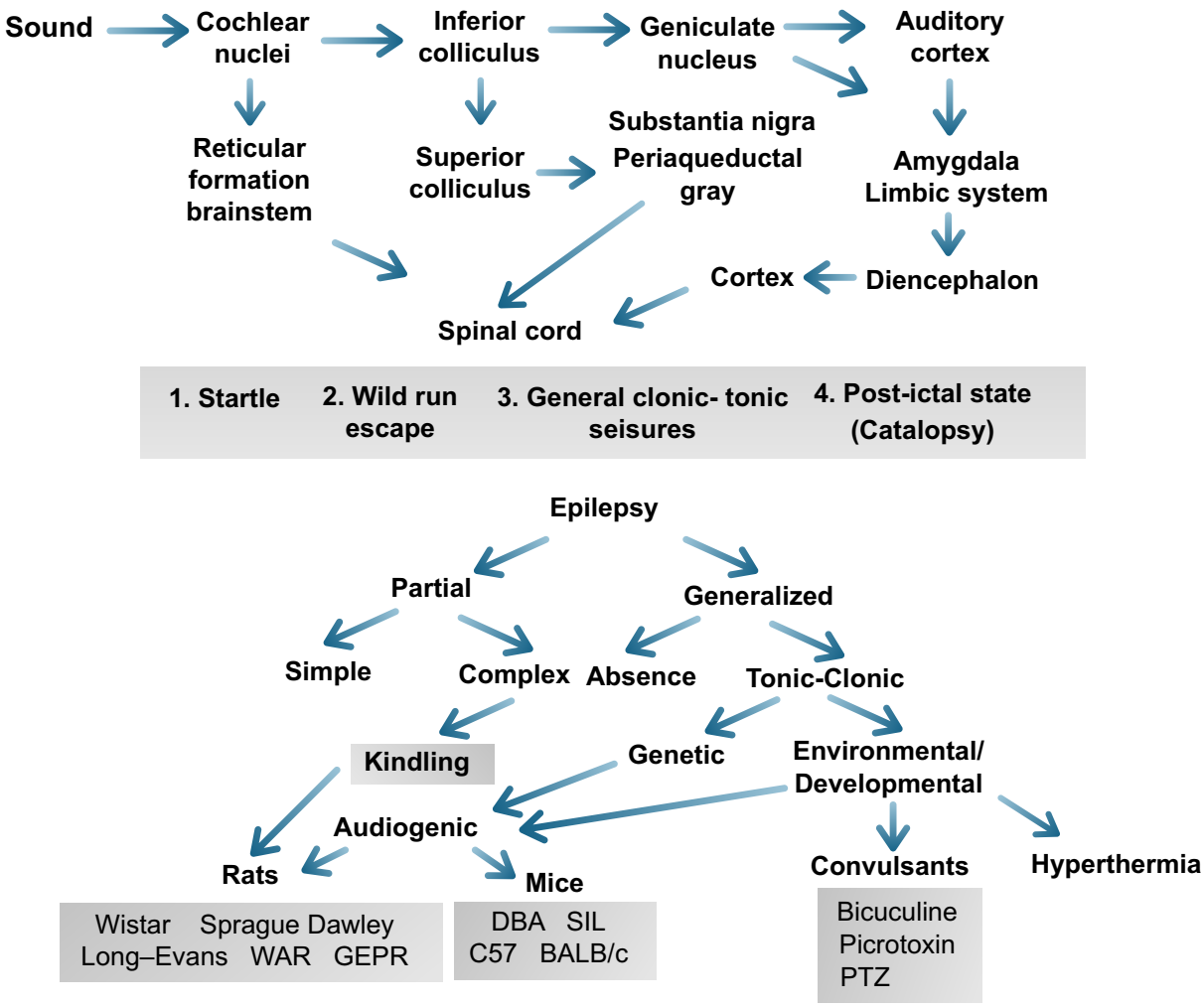

Fig.1. Pathways in audiogenic epilepsy and the models of epilepsy.

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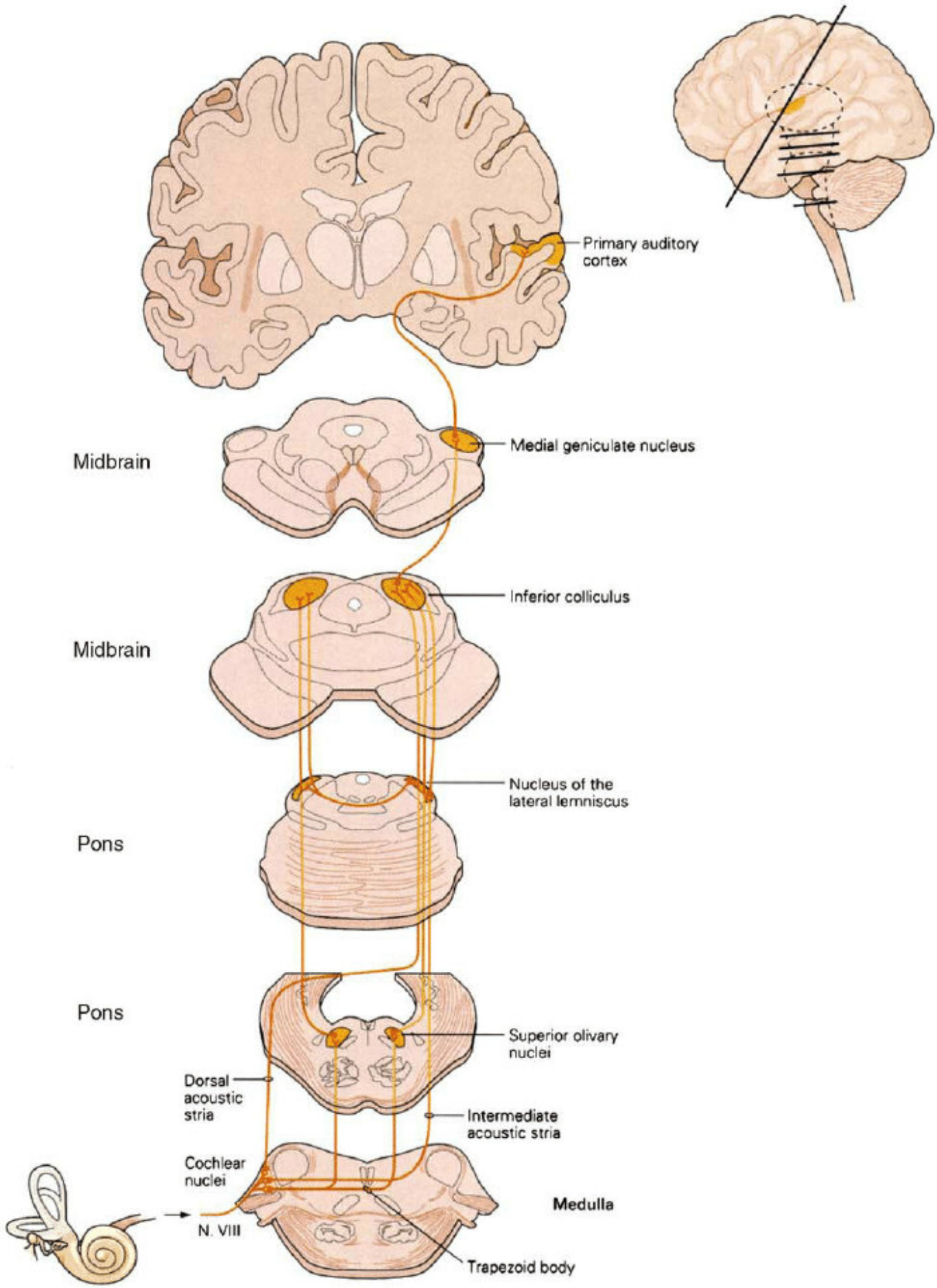

Fig. 2. The central auditory pathways extend from the cochlear nucleus to the auditory cortex. Based on Graven, S.N. Browne, J.: Auditory Development in the Fetus and Infant. Newborn and Infant Nursing Reviews 8(4):187-193, 2008 (Resarch gate)