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KRS: 0000628361
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USE OF POLYMER INCLUSION MEMBRANES FOR METALS RECOVERY

Daria Bożejewicz
Faculty of Mechanical Engineering University of Sciences and Technology
Prof. S. Kaliskiego 7, PL85796 Bydgoszcz, Poland
corresponding author: dariabozejewicz@wp.pl

Abstract

The growth of industrial development significantly contributes to the improvement of living comfort, however, the problem of the waste generation being a result of technological processes is still a problem of the modern world. Metal-bearing waste is among the strategic raw materials due to the possibility of their reuse, in fact natural deposits of these raw materials are protected, and it reduces the amount of waste present in landfills. Now, effective methods are being sought for the recovery of non-ferrous metals from industrial waste, using, inter alia, membrane processes (e.g. PIM). In the polymer inclusion membranes use a lot of ion carriers. Methods are being sought to understand the processes that take place inside the membrane. Detailed analysis of the resulting metal ion-carrier complex, it may be useful to use high-resolution mass spectrometry methods and tandem mass spectrometry.

Keywords

Polymer inclusion membranes (PIMs), ions carriers, high-resolution mass spectrometry, tandem mass spectrometry, recovery of metals

Introduction

Industrial waste contains, among others a lot of metals, including heavy metals. Depending on the industry, various types of pollution can be found in waste, both harmful to human and animal health, as well as useful for economic reasons. We have different types of pollution that can be found in the waste depending on the kind of industry. For example it compounds harmful substances to human and animal health and it also compounds useful substances for economic reasons. In case of metals such as zinc, copper, cadmium, nickel, gold etc. one should look for more and more effective methods of their recovery. It is really important in environmental and also economic aspects, because metal prices are oscillating in amounts of several thousand dollars, depending on the current market and political situation.

According to the document of the Central Statistical Office '*Environment Protection 2017'* in 2016 Poland generated 140 million tons of waste, including 8% of municipal waste (i.e. 12 million tons). The main source of waste generated in 2016 was mining and quarrying, who generated about 52%, while industrial processing about 21%, as well as production and supply of electricity about 16% of the total waste produced (Figure 1). Considering the last ten years, the highest volume of

waste generated was the waste generated during the exploration of mining and physical and chemical processing of ores and other minerals. In 2016 it accounted for 56% of the total waste generated, and waste from thermal processes accounted for 22% [1].

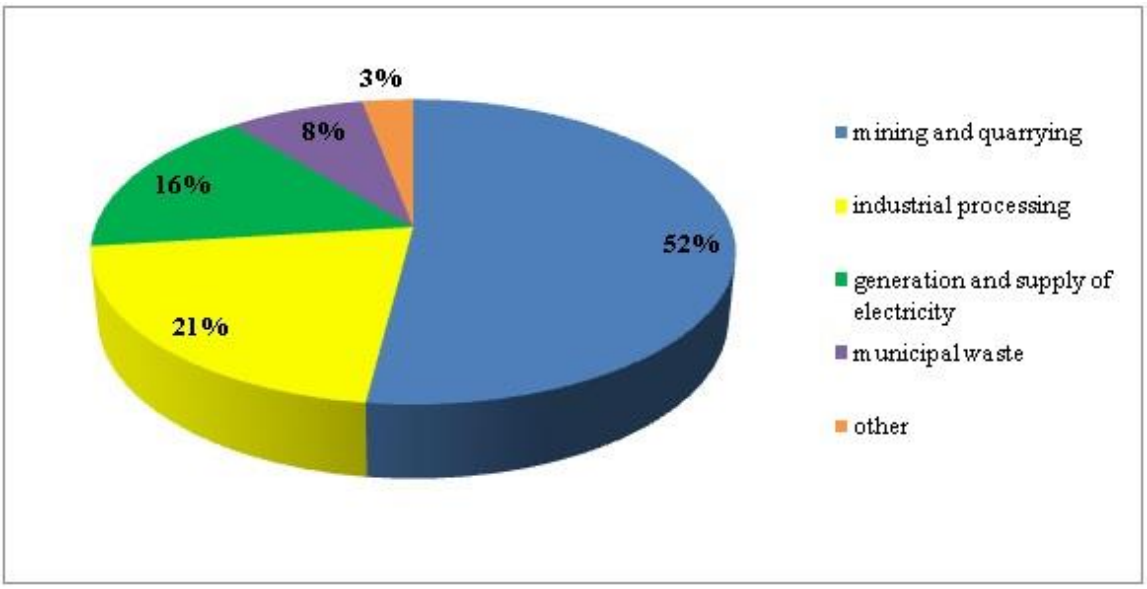

Figure 1. Percentage of waste generated in 2016 based on the study by D. Bochenek, Environmental Protection Environment 2017, October 2017
Source: own elaboration

On the basis of data available in the GUS document '*Environment 2017'*, 49% of waste was subjected to recovery, 42% was disposed of via storage, and the remaining 4% of waste was disposed of in a different way (Figure 2) [1]. The most important role in the process of waste disposal is played by the recovery process, which allows the subsequent use of raw materials. Nevertheless, the storage process is still a big problem in Poland. Storage is an ineffective process that does not bring economic and environmental benefits.

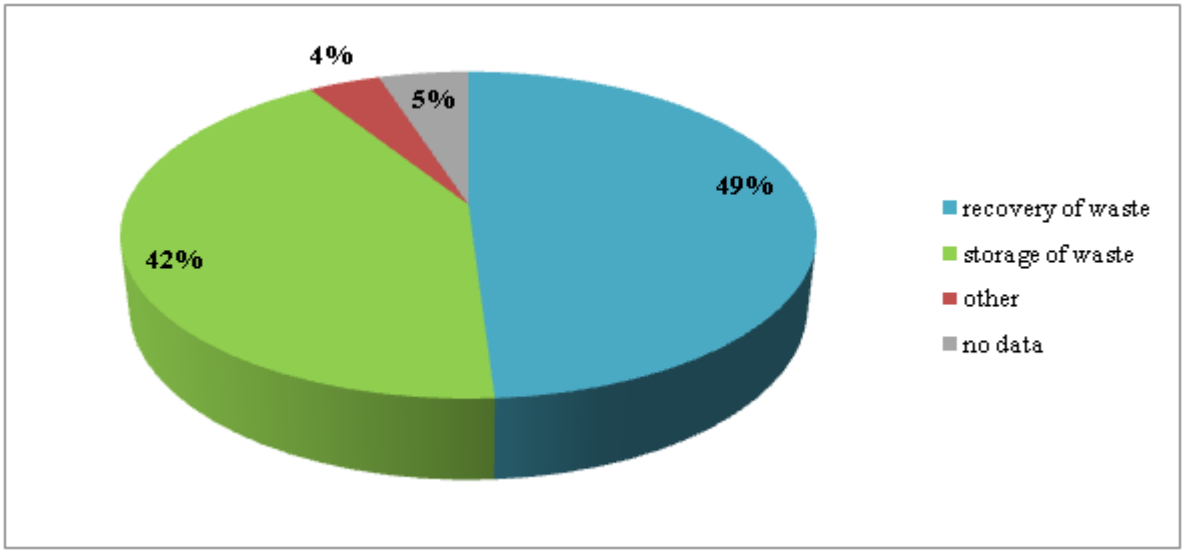

Figure 2. Percentage of waste generated in 2016 based on the study by D. Bochenek, Environmental Protection Environment 2017, October 2017
Soruce: own elaboration

Due to the large amount of waste generated, it becomes important to find an effective method of recovery of non-ferrous metals present in waste, with a view to their further use in industrial processes. The methods of metal recovery can be divided into the recovery of metals from aqueous liquid solutions and from constant precipitation. There are many known methods of the separation of metal ions from aqueous solutions. Since the end of the 20th century, membrane processes have been of increasing interest among scientists and technologists, enabling metal recovery at low concentrations. In addition, they can be used in new and already operating industrial installations. The main advantage of membrane processes is the ability to conduct separation in a continuous manner with a low energy demand and low technological costs.

In the processes of selective separation and recovery of heavy metal ions, polymer inclusion membranes (PIMs) were used. A characteristic feature of polymer inclusion membranes is the high stability during the transport of metal ions, where the carrier, which is the complexing compound, is responsible for the binding and transport of metal ions through the membrane. The polymer plays a key role in ensuring the mechanical strength of the PIM-type membrane, and its properties significantly affect the permeability and durability of the membranes [2].

Polymer inclusion membrane

The most popular, polymer inclusion membranes (PIMs) consists of an ion transporter, a polymer matrix (usually polyvinyl chloride (PVC) or cellulose triacetate (CTA)), as well as a plasticizer [2]. One method of preparation of a polymer inclusion membrane is the dissolution of appropriate amounts (e.g., % by weight) of a polymer, e.g. PVC and a suitable ion transporter. The obtained solution is then poured into a glass ring e.g. 7.5 cm in diameter, which is placed on a flat glass plate previously cleaned with acetone. The mixture is covered with a filter paper and a watch glass and it is left to stand for 24 hours to evaporate the solvent [3]. Most polymer inclusion membranes are obtained in the form of casting flat membranes. Almeida indicates the possibility of obtaining PIMs by filling a coated PIM column. This method consists in preparing a cylindrical PIM with an empty agent by passing the membrane components, which are then cast on a glass capillary tube [4]. Polymer inclusion membranes prepared in this way can be used in the separation process. Transport of metal ions through polymer inclusion membranes is possible due to the use of an appropriate ion transporter.

In the polymer inclusion membranes, the selection of an appropriate metal ion carrier is very important. In choosing a metal ion carrier, knowledge about the properties of carriers and separated metal is necessary. When selecting the ion carrier, it is important to remember that the basis for the polymer inclusion membranes is the complexation reaction. This reaction occurs due to the presence of a free electron pair on the non-ligand binding orbitals and empty orbitals present in the central atom that are able to absorb the electron pairs of these ligands [5]. In polymer inclusion membranes, metal ion transporters can be organic chemicals that are well soluble in the membrane, but do not dissolve in aqueous solutions. It is also important that the carrier used is characterized by selectivity and reversible interaction with the separated metal ion [6]. During the separation of non-ferrous metal ions, the frequently used carriers in polymeric non-ferrous membranes include D_2EHPA (di-(2-ethylhexyl) phosphoric acid), Cyanex 272 (di-(2,4,4-trimethylpentyl) phosphonic acid), β-diketone derivatives [7-9].

The D_2EHPA acid (Figure 3) in PIMs is used to separate not only zinc, but also manganese, nickel, copper, etc. Wang and co-authors for the recovery of non-ferrous metals (between zinc, magnesium) use polymer inclusion membranes containing LIX 84I ion carriers (2-hydroxy-5-nonyl acetophenone oxime) and D_2EHPA (di (2-ethylhexyl) phosphoric acid), thus proposing a PIM double membrane system for simultaneous separation and enrichment [10]. In addition, D_2EHPA acid is used as a carrier to remove selectively Fe (III) ions from acidic aqueous solutions [11].

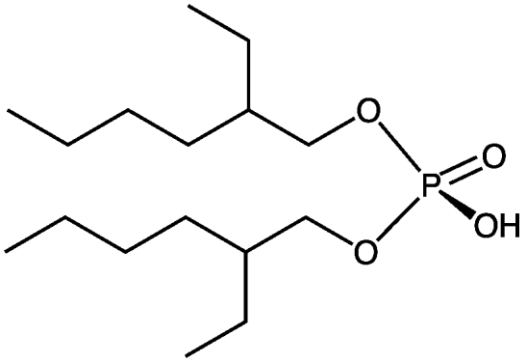

Figure 3. The chemical formula of D_2EHPA, used as an ion transporter in polymer inclusion membranes
Source: own elaboration

The next frequently used carrier in PIMs is Cyanex 272, which introduced into the CTA polymer matrix, will enable efficient transport of zinc ions. Yilmaz and co-authors have found that the Zn(II) transport by PIM is affected by variables such as the initial pH and the concentration of zinc ions of the supply phase and the concentration of the ion transporter (Cyanex 272) and the receiving phase [8]. Besides mentioned above ions metals D_2EHPA and Cyanex 272 also can use to palladium separation. According to Bonggotgetsakul, the production of the nanoparticles palladium PdNP used D_2EHPA as ions carriers, in order to maximum surface coverage polymer inclusion membrane PdNP with an average size of 38 nm [3].

And the next ions carriers are β-diketones derivatives (Figure 4), which specific construction i.e. the presence of two carbonyl groups and a methylene group per molecule, can use to transport a lot of metals including zinc, copper, cobalt or nickel. On the basis of the research carried out, Witt and co-authors found that the increase in roughness increases the porosity of the membrane. These parameters are very important in transport processes ions, because the greater the roughness, the larger the active surface of the membrane. Ions Zn(II) are transferred in supply phases to receiving phases with higher speed than ions Cu(II) and Ni(II) and depends on the type of ions carriers [12].

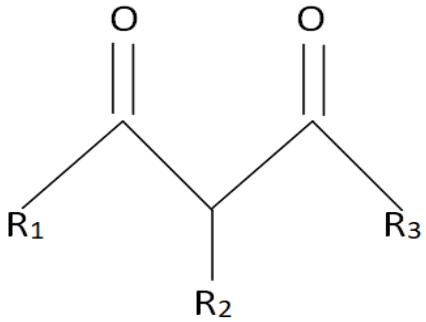

Figure 4. Chemical formula of β-diketone molecule, used as an ion transporter in polymer inclusion membranes
Source: own elaboration

Separated ammonia and calcium, lead or zinc ions from aqueous environmental solutions has been with used dinonyl naphthalenesulfonic acid (DNNS) [13]. Recovered are also the waste containing precious metals, among others from old electronic equipments, for example the mobile phones.

Kubota with co-authors points to the newly synthesized D_2EHAG extractant (N-[N,N-di-(2-ethylhexyl)aminocarbonylmethyl]glycine), which is characterized by high selectivity to Au (III) ions with respect to other metals present in solutions [14]. Nanoparticles gold can be made with polymer inclusion membranes on based polychloride vinyl and commercial ions carriers i.e. Aliquat 336. In this case through the reduction of gold(III) it is extracted into the membrane as a complex $[AuCl_4]^-$[15].

The obtained results confirm the effectiveness and rightness of using ion transporters, including such as: D_2EHPA acid, commercial Cyanex 272 or β-diketone derivative. Own research and review of specialist literature prove that polymer inclusion membranes are able to transport metal cations with very good yield and high selectivity. The PIMs studies contain mentioned above derivatives as metals ions carriers that provide the necessary information on the speed of metal transport by PIMs, process selectivity, effectiveness, etc. However, this research does not provide information of the resulting bonds between the recovered metal and the organic ion carrier. A closer understanding of the processes taking place inside the membrane may be helpful in further design of the technological process. For this purpose, mass spectrometry and tandem mass spectrometry methods can be used to determine the resulting complex between the metal and the ligand.

Mass spectrometry

Mass spectrometry (MS) is an analytical tool used to measure molecular weight and analysis of organic compounds. This method allows measurements at very low concentrations. Mass spectrometry methods can be divided into so-called soft and hard. In case of "soft" ionization, a molecular ion is formed, without fragmentation of the molecule, for example, the ESI, MALDI method. In contrast, in the "hard" ionization there is a fragmentation of the studied molecule, e.g. EI, CI. Although mass spectrometers differ from each other by ionization of molecules and separation of ions, all spectrometers operate according to one principle (Figure 5) [16].

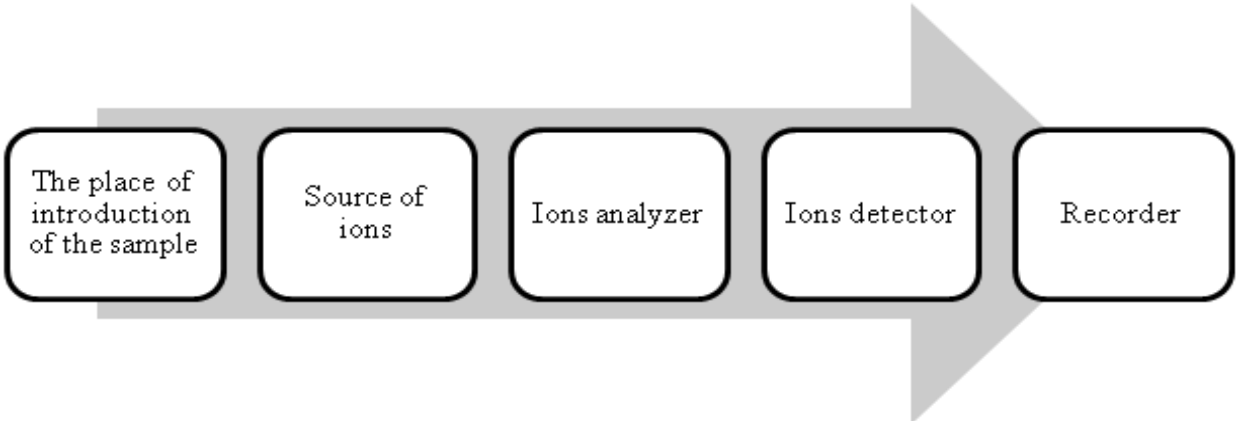

Figure 5 Block diagram of the mass spectrometer operation
Source: own elaboration

Mass spectrometry allows you to get complete information about a given compound when it is in a clean state, because the spectra of contaminated compounds are difficult to interpret. For this reason, the MS method should be accompanied by a pre-separation method. Coupled techniques play an extremely important role here. In this way, the following methods were developed: GC-MS, HPLC-MS and HPCE-MS, which combine the mass spectrometer with a gas chromatograph, high-performance liquid chromatography or high-performance capillary electrophoresis [16]. Another option is to introduce a so-called tandem mass spectrometry. This method (marked with the MS/MS symbol) consists of performing controlled ion decay of the tested compound and further analysis of the mass of the obtained fragments, i.e. the ratio of mass to ion charge m/z [17]. Tandem mass spectrometry is used to identify as well as determine the structure of simple chemical compounds, which provides information about the structure and type of fragmentation ions and how to link them. The choice of the appropriate method among the many known methods of fragmentation depends on the properties of the test compound and on the construction and parameters of the mass spectrometer [18].

The structure of macromolecular compounds is investigated by means of mass spectrometry (MS) and tandem mass spectrometry (MS/MS) methods. One of the main methods is used in the analysis of polymers is MALDI type spectrometry (laser desorption with the use of a matrix). This method provides a number of information about the chemical structure of the polymer, while allowing the identification of chains, end groups, functions and provides information about the presence of other fragments present in the structure. Thanks to the use of MALDI spectrometry during the analysis of macromolecular compounds, it is possible to examine the resulting structure or develop a further research procedure. In case of synthetic polymers analysis it's often used MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry) i.e. laser desorption with the use of a matrix with measurement of ion time [19].

Since last years, an increasingly common method of ionization is the electrospray (ESI) method, which has been used in the analysis of both low molecular weight compounds and macromolecules, such as biopolymers or complex complex systems. The electrospray ionization technique is one of the mildest methods in spectrometry, because the resulting ions are characterized by a small excess of internal energy. The ESI technique has found particular application in the analysis of chiral compounds. Drabik underlines that the electrospray technique can be successfully use to analyze chiral compounds, where the relative intensity of signals of the analyzed spectrum depends on the enantiomeric composition of the analyte under study [20]. Nevertheless, experimental studies confirm that proper results are obtained only by using several spectrometry methods simultaneously.

Summary

Industrial waste is a very big problem due to environmental and economic reasons. One of metal separation method in aqueous solution is membranes processes, which is characterized as a higher efficiency process, with relatively low financial and technological costs, and the metals recovered in this way can be further used in industry. One of the membranes techniques is the polymer inclusion membranes. In the PIMs for the separation non-ferrous metals can be used among the following metal ion carriers: D_2EHPA, Cyanex 272 or β-diketones derivatives, whereas to recover precious metals (e.g. gold and palladium) commercial ion carriers i.e. Cyanex 272 or

Alquat 336 can be used. The transport and selection of the separation process using PIMs is well known and studied by many researchers, which is possible to effectively control the separation process and select the most effective metal recovery medium that we want to receive. This raises one question, if during examining the structure of the complexes created inside the membrane, will it affect even better process efficiency? For this purpose, it may be appropriate to use high-resolution mass spectrometry methods and tandem mass spectrometry. These methods are becoming more and more popular in the analysis of macromolecular compounds, in polymer chemistry or in purification processes, therefore, PIMs may be used during the study. In-depth analysis of the structures created inside the membrane between the ligand and the separated metal will allow you to learn more about the process.

Conclusion

Polymer inclusion membranes, due to the high selectivity of the process and the availability of many ion transporters, are becoming more and more popular among researchers and engineers. The researches on the PIMs confirm their effectiveness and possibility in using in industry for recovery metals in aqueous solution, and efficiency transport depends on using ion carriers. Depending on the separation conditions, many metal ion carriers are available, and so during the recovery of non-ferrous metals, D_2EHPA, Cyanex 272 and β-diketone derivatives and are proved to be effective carriers during recovery of precious metals Cyanex 272 and Aliquat 336. Analyzing the available literature data, one can conclude that the methods of high resolution mass spectrometry and tandem mass (MS/MS methods) can provide information about the structure and elemental composition of the resulting complexes, therefore the use of these methods seems to be right in order to better understand the processes occurring inside the membrane, primarily metal-ligand connections.

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MEASUREMENTS EMISSIONS OF POLLUTANT-GAS IN POWER UNITS

Aleksandra Czajkowska
Faculty of Mechanical Engineering, University of Science and Technology in Bydgoszcz
corresponding author: czajkowska.aleksandra91@gmail.com

Abstract:

The Energy Policy is developed by the European Commission for countries belonging to the European Union, activities that accomplished the goals were included Green Paper. Goals concern the reduction of harmful greenhouse gases until to 2020 at least 20% compared to 1990. Determination the value of efficiency cleaning the fumes from power boilers, it is realized by measured concentrations of individual chemical compounds at the inlet of the electrostatic precipitator and at the outlet of the device. The gravimetric method is most often used in the energy industry to control efficiency the process dedusting exhaust fumes, Researches realized according with the Polish Standard PN-Z-04030-7: 1994 „Measurement of the concentration and mass flux of dust in exhaust gases by the gravimetric method".

Keywords:

power units, electrostatic precipitators, dust measuring devices, dedusting exhaust fumes, environment protection

Introduction

The result of production the energy into power boilers are pollutions. Into the dusting medium are included chemical compounds as: oxides (O_2), in particular carbon monoxide (CO), sulfur dioxide (SO_2), carbon dioxide (CO_2), nitrogen dioxide (NO_2), hydrogen fluoride (HF) and carbon tetrachloride (CCl_4).

Phenomena enabling the purification of flue gases, which correct use allows their subsequent use during defining the mechanism of the electrostatic precipitator, included: electrostatics, mechanics of aerosols or thermodynamics [3].

Gravimetric measurements are used to check the efficiency of the dust removal process, currently in the energy industry, in particular in conventional power plants [1]. Researches are realized during the warranty period and on behalf of the investor after the warranty period. The correctness of the process of dedusting exhaust gases from dust are diagnosed according with the Polish Standard PN-Z-04030-7: 1994 „Measurement of the concentration and mass flux of dust in exhaust gases by the gravimetric method" [4, 13].

Emission standards of NO_x, SO_x and dust

Currently environmental indicators relate mainly to control three parameters [2, 11, 12]. In current regulations are included acceptable values NO_x, SO_x and dust emissions to environment depending on power of the boiler. In regulations of Environment Minister are appeared decrees referring to the type of combustion devices [11, 12]. The following tables refer to the emission standards for combustion plants where the total rated thermal input is equal to or more than 50 MW. Emission standards of NO_x, SO_x and dust are included in the regulation of Environment Minister in Poland of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities" they refer to functionality [11].

Table 1. Emission standards for sulfur dioxide at content 6% oxygen in exhaust gases

Nominal thermal power of the source [MW]	Emission standards for sulfur dioxide [mg/m^3]			
	at content *6%* oxygen in exhaust gases			at content *3%* oxygen in exhaust gases
	the biomass	the peat	other solid fuels	the liquid fuels
1	*2*	3	4	5
≥ 50 and ≤ 100	200	300	400	350
> 100 and ≤ 300	200	300	250	250
> 300	200	200	200	200

Source: The regulation of Poland Environment Minister of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities"

Table 2. Emission standards for sulfur dioxide at content 3% oxygen in exhaust gases

Type of gas	Emission standards for sulfur dioxide [mg/m^3] at content 3% oxygen in exhaust gases
1	2
the liquid gas	5
low calorific coke oven gas	400
low calorific blast furnace gas	200
other gas	35

Source: The regulation of Poland Environment Minister of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities"

Table 3. Emission standards for nitrogen oxides at content 6% oxygen in exhaust gases

Nominal thermal power of the source [MW]	Emission standards for nitrogen oxides [mg/m^3]		
	at content 6% oxygen in exhaust gases		at content 3% oxygen in exhaust gases
	the biomass and the peat	other solid fuels	the liquid fuels
1	2	3	4
≥ 50 and ≤ 100	300	300, 450 - in the case of burning lignite dust	450
> 100 and ≤ 300	250	200	200
> 300	200	200	150

Source: The regulation of Poland Environment Minister of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities"

Table 4. Emission standards for nitrogen oxides and particular carbon monoxide at content 3% oxygen in exhaust gases

Substance	Emission standards for nitrogen oxides and particular carbon monoxide [mg/m^3], at content 3% oxygen in exhaust gases		
	Natural gas	Coke oven gas, blast furnace gas, low caloric gas	Other gas
1	2	3	**4**
Nitrogen oxides	100	200	200
Particular carbon monoxide	100	-	-

Source: The regulation of Poland Environment Minister of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities"

Table 5. Dust emission standards at content 6% oxygen in exhaust gases

Nominal thermal power of the source [MW]	Emission standards for dust [mg/m^3]		
	at content 6% oxygen in exhaust gases		at content 6% oxygen in exhaust gases for gas turbines – 15%, for other sources – 3%
	the biomass and the peat	other solid fuels	the liquid fuels
1	2	3	4
≥ 50 and ≤ 100	30	30	30
> 100 and ≤ 300	20	25	25
> 300	20	20	20

Source: The regulation of Poland Environment Minister of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities"

Table 6. Dust emission standards at content oxygen in exhaust gases for gas turbines – 15%, for other sources – 3%

Type of gas	Emission standards for dust [mg/m^3], at content 6% oxygen in exhaust gases for gas turbines - 15%, for other sources - 3%
1	2
blast furnace gas	10
gases produced by the steel industry that may be utilize elsewhere	30
other gas	5

Source: The regulation of Poland Environment Minister of 4th November 2014 on „The emission standards for certain types of installation, fuel combustion plants and waste incineration or co-incineration facilities"

As previously mentioned, currently and mainly are controlled values, which are included in regulations of Environment Minister, relating to combustion devices so, chemical compounds such as: NO_x, SO_x and additionally the value of dedusting exhaust fumes from a dust [5-10].

Laboratories, dealing with measurements, researching the processes, schemes and the energy devices, they have own list of offered parameters, which could research during accomplish the control. However the final selection of the measured quantities belongs to the person, who order the accomplishment test. Commutation the measured indicators of the pollutant-gas emission are realized by following formulas (the reference method – SRM):

$$C_{USR} = C_m \times \frac{T_m}{T_{ref}} \times \frac{p_{ref}}{p_m} \times \frac{100}{(100 - Wg)} \times \frac{(21 - O_{2ref})}{(21 - O_{2m})} \quad (1)$$

where:

C_{USR} – concentration under reference conditions;

Cm – concentration under conditions of temperature, pressure, humidity and oxygen content in the measurement cross-section;

V_{USR} – the flowrate under reference conditions;

V_m – the flowrate under conditions of temperature, pressure, humidity and oxygen content in the measurement cross-section;

T_m – the temperature in the measurement cross-section;

T_{ref} – the referece temperature = 273,15 K;

p_m – absolute pressure in the measurement cross-section;

p_{ref} – the reference pressure = 1013,25 hPa;

W_g – moisture content in the measurement cross-section;

O_{2m} – oxygen content in the measurement cross-section;

O_{2ref} – oxygen content reference.

The following formula is used in case of necessity correction the drift (D_{ryf}) of instrumentally measured concentration the pollutant-gas values emissions:

$$C_{kor} = \frac{C-(B(t_0)+Dryf(B)\times t)}{(A(t_0)+Dryf(A)\times t)} \quad (2)$$

where:

C_{kor} – corrected concentration;

C – measured concentration;

$A(t_0)$ – (the result from the analyzer after adjustment in time (t_0) in the scope point - the result from the analyzer after adjustment in time t_0 at the zero point) / (calibration gas concentration in the scope point - calibration gas concentration at the zero point);

$B(t_0)$ – the result from the analyzer after adjustment in time t_0 at the zero point;

$D_{ryf}(A)$ – {[(the result from the analyzer while checking d_{ryfu} in time t_{end} in the scope point - the result from the analyzer while checking d_{ryfu} in time t_{end} at the zero point) / (calibration gas concentration in the scope point - calibration gas concentration at the zero point)] - $A(t_0)$} / ($t_{end} - t_0$);

$D_{ryf}(B)$ – (the result from the analyzer while checking d_{ryfu} in time t_{end} in the scope point - the result from the analyzer while checking d_{ryfu} in time t_{end} at the zero point) / ($t_{end} - t_0$);

$t_{end} - t_0$ – time of the measurement period in minutes (the period between adjustment and verification the drift at the end of the measurement period).

Measurement method

Measurements of concentrations the exhaust gas components

Researches of concentrations NO_x, CO, CO_2 and O_2 are realized continuously by instrumental methods, using a multi-gas analyzer:

- NO_x – PN-EN 14792:2017-04;
- CO – PN-EN 15058:2017-04;
- CO_2 – ISO 12039:2001;
- O_2 –PN-EN 14789:2017-04.

Researches of concentrations SO_2 are realized by manual method according to PN-EN 14791:2017-04, instead researches of the steam are performed by manual method according to PN-EN 14790:2017-04.

The heated probe, heated hoses and particulate filter are used to suck the exhaust sample. The extraction method is used for parallel measurements, there is a need to separate the dust contained in the exhaust fumes. Initial cleaning of exhaust fumes takes place in heated filter mounted at the outer end of the probe. Exhaust gas free from dust is transported by heated hoses:

a) for the needs of NO_x, CO, CO_2 and O_2 measurement– the diaphragm pump with heated-head. Then the exhaust ges is steered to special construction (Jet-stream), a coller removing the steam contained in the gas – the sample temperature at the outlet of cooling block is maintained by the controller at the set level ≤ 4°C. Dry, clean exhaust gas is steered to the analyzer where the appropriate concentration measurement takes place.

b) for the needs of SO_2 measurement – a set for manual measurements, which is compound with heated-box and two scrubber contains absorbing (r-r), and the precise gas volume meter to determine the exhaust gas stream volume.

c) for the needs of steam measurement – a set for manual measurements, which is compound with box with condensing scrubber and with an adsorption degree, and the precise gas volume meter to determine the exhaust gas stream volume.

Measurements of the volume flow and dust pollination concentration

Researches of the exhaust gas stream are realized according to PN-Z/04030-7: 1994. For this purpose it is used multi-point probing to define the dynamic pressure of gases by micromanometer and tube type „S".

Researches of the exhaust gas stream are realized according to PN-EN 13284-1:2007. Similarly as for flow, sampling is carried out in multipoint, in the grid designated for the flow probing. In this method is used a gravimetric dust meter, which is consists of the aspiration probe with dust separator and with with exchangeable tips, calibrated measuring orifice with built-in thermometer, moisture separator with temperature measurement, and a set of differential manometers, and the extractor for exhaust suction.

The airtightness of the dust meter devices is realized, each time before a sample is taken, to avoid sucking in air from the environment. Temperature of the test and filtration of the exhaust gas sample have to be kept at a level 160°C, while maintaining the condition isokinetic probe.

The temperatures are measured by sensors. Static pressure in the measurement cross-section and differential pressures on the measuring orifice is estimated by differential pressure gauges type U- tube. The low pressure on the measuring orifice of dust meter devices is estimated by a vacuometr.

The mass of the captured material during aspiration, is determined on the analytical balance by the same procedure for filters and with the collected material. Quartz, flat filters, before aspiration is conditioned at temperature 180°C. Measuring filters before aspiration, is conditioned at temperature 160°C.

Measurement of the moisture the exhaust gases

Measurements of moisture content are realized by condensing and temperature method by measuring the condensed moisture from sucked in partial gas stream and by measuring the temperature of saturation in the dehydrator. The amount of moisture captured is measured by the measuring cylinder according to PN-EN 14790:2017-04, which replaced pre-existing norm PN-EN14790:2006.

The European Standard describes the standard method (SRM) using a gas sampling system and condensing-adsorption technique, which are used to determine the concentration of water vapor into exhaust gas emitted to atmospheric air through wires and chimneys [14]. In the norm PN-EN 14790:2017-04 performance characteristics were determined, what should be specified and criteria for action, what should be fulfilled for measuring systems, in which this method of measurement is used [14].

Is used for periodic measurements and for calibration or control the automatic measuring systems (AMS), which are permanently mounted on the chimneys. Mainly AMS used for checking the limit of emissions [14].

The standard gives the criteria demonstration of equivalence of alternative methods to the standard reference method with the use EN 14793:2017. It is used in the range of water vapor

content expressed as the volume fraction from 4% to 40% and in the range of water vapor mass concentration from 29 g/m^3 to 250 g/m^3 into a moist gas, although at a given temperature the upper limit of applying the method is related to maximum steam pressure in the air or in gas. In this standard all concentrations are expressed for contractual terms (273 K and 101,3 kPa) [14].

Determination the chemical composition of fly ash samples

Determination of the content of trace elements in samples of emitted fly ash is carried out on the emission spectrometer. Determination of trace elements in the samples by the laboratories perform according to the standard PN-EN 14385:2005 [15].

The standard contains information about the reference manual method, determination of the mass concentration of special elements in terms from 0,005 to 0,5 mg/m^3, in the off-gases from hazardous and municipal waste incineration plants [15].

Special elements are: antimony (Sb), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), tal (Tl) and vanadium (V). The method can also be used to determine mercury (Hg), when sampling from the side stream is according with EN 13211. The method can also be used to with reference to the off-gases from other sources if they have a composition similar to the following [15]:

- total particulate matter from 0 to 20 mg/m^3;
- TOC from 0 to 20 mg/m^3;
- HCl from 0 to 20 mg/m^3;
- HF from 0 to 2 mg/m^3;
- SO_2 from 0 to 100 mg/m^3;
- NO_x from 0 to 500 mg/m^3 (jako NO_2);
- CO from 0 to 250 mg/m^3;
- CO_2 from 0 to 15 procent (suchy, rzeczywisty);
- H_2O (g) from 10 to 35 volume percent, O_2 from 3 to 17 percent (dry, real).

Determination of mercury vapor in exhaust gases

Collection of exhaust gas samples and determination of total mercury in the exhaust gases in the form of the gas mercury is realized according to PN-EN 13211:2006. The scope of the standard includes the reference manual method and determination of mass mercury concentration, in the range from 0,001 to 0,5 mg/m^3, in the off-gases from canals and chimneys or from other sources if they have a composition similar to the following [16]:

- total particulate matter from 0 to 20 mg/m^3;
- C_xH_y from 0 to10 mg/m^3;
- HCl from 0 to 50 mg/m^3;
- HF from 0 to10 mg/m3;
- SO_2 from 0 to 250 mg/m^3;
- NO_x from 0 to 500 mg/m^3;
- CO_2 from 0 to 15% objętości;
- H_2O (g) from 10 to 25% volume (real);
- O_2 from 8 to 15% volume (dry, real), temperature from 60 to 140°C.

Flue gas samples are absorbed into absorption solutions, after initial separation on filters with mercury absorbed on the surface of the dust (constant sample). Determination of mercury content (constant sample) is carried out by atomic absorption spectrometry [16].

Determination of PM10 and PM2,5 fractions

Researches of the particle size distribution emitted dust (mean particle size), is carried out by laser diffraction method according to ISO 13320:2009 [17].

ISO 13320:2009 provides guidance on instrument qualification and size distribution measurement of particles in many two-phase systems (e.g. powders, sprays, aerosols, suspensions, emulsions and gas bubbles in liquids) through the analysis of their light-scattering properties. It does not address the specific requirements of particle size measurement of specific materials [17].

ISO 13320:2009 is applicable to particle sizes ranging from approximately 0,1 μm to 3 mm. With special instrumentation and conditions, the applicable size range can be extended above 3 mm and below 0,1 μm [17].

For non-spherical particles, a size distribution is reported, where the predicted scattering pattern for the volumetric sum of spherical particles matches the measured scattering pattern. This is because the technique assumes a spherical particle shape in its optical model. The resulting particle size distribution is different from that obtained by methods based on other physical principles (e.g. sedimentation, sieving) [17].

Summary

This work aimed at gathering methods and standards, which are used when determining the size of dust-gas particles. Measurements are carried out by laboratories specializing in research for a group of plants related to the power industry. The measurements are mainly aimed at determining the level emitted pollutants in the exhaust gas, taking into account the limit values contained in Regulations of the Environment Minister and in the indicated Polish standards. The captured limit values depend mainly on two factors, from the power boiler and from the fuel used.

The methodology of the conducted research is changed with the tightening of EU regulations relate to environmental protection. It is extremely important to monitor the indicated parameters due to the environment and on the impact of individual chemical elements on individual components, subassemblies devices included in the composition power units. Researches are carried out cyclically during the warranty period and on behalf of the investor after the warranty period. Calculated dust-gas particle sizes must be archived and included in research reports.

In the next step should think about adding weights of individual parameters to the reports on the measurements implementation, in order to systematize the dust-gas values in terms their criticality for the environment and machines components in power units, taking into account using the type of fuel.

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MODELLING DESIRED BEHAVIOUR PATTERNS IN MEERKATS (*SURICATA SURICATTA*) IN THE ZOO

Monika Lik*, Marcelina Dukacz, Magdalena Węglarz
Rada Naukowo-Dydaktyczna, Leśny Park Kultury i Wypoczynku, Myślęcinek, spółka z.o.o.,
ul. Gdańska 173-175, 85-674 Bydgoszcz
*corresponding author: lik.m@tlen.pl

Abstract:

The aim of this paper was to develop protocols of procedure which would result in obtaining desired reactions in animals, namely the desired behaviour of meerkats kept in Bydgoszcz Zoo. Behavioural training, which was intended to result in modelling 2 behavioural patterns was conducted in group of 4 individuals - 3 females and 1 male of meerkats making up one herd. Modelling of behavioural patterns was carried out only with the positive reinforcement technique. Behavioural pattern modelling activity was divided into 4 stages leading to the obtainment of desired behaviour: initiation of desired reactions, positive reinforcement of reaction, subduing undesired reactions in the presence of de-motivator, consolidating the desired reactions by prolonging the exposure to the stimulus in the presence of a motivator. Behavioural pattern modelling of animals living in zoos positively affects their welfare, reducing stress related to breeding procedures, caring and veterinary activities.

Keywords:

behaviour patterns, meerkats, positive reinforcement

Introduction

Techniques of behavioural pattern modelling consist in making use of stimuli which motivate animals to display behaviour expected of them by behavioural trainer. Motivators are based on animals' behaviour and their instinctual drives such as need to search for food and to explore. De-motivators are used in order to subdue the displayed (unwanted) behaviour, which in case of positive reinforcement training mean lack of motivators. Environmental elements considered to be strong de-motivating stimuli are those which trigger defensive reactions in animals or their self-preservation reactions stemming from e.g: the need to flee, seek refuge or attack. Schemes of procedures (protocols) including motivators and de-motivators result in the animal's expected behaviour. They are used in animals serving in uniformed and civil forces, in animals working in sport, therapy, laboratories and in exotic animal species kept in zoos [1,2,3,4,5,6,7,8].

The basic method of animal training is so called positive reinforcement method. The training is based on the cooperation between the trainer and the animal without the use of force towards the animal and with simultaneous rewarding their desired reactions. The reward may be a treat, a toy or a tactile stimulus in the form of stroking or patting the animal as well as a verbal appreciation [2,8].

Positive reinforcement techniques make it possible to evoke specific behaviour even in entire herds of exotic animals kept in zoos, e.g: moving whole groups of animals from one enclosure to another.

Being familiar with behavioural techniques, i.e. techniques based on instinctive behaviour of the animal species a person works with enables one to develop a pattern of procedure in breeding works, veterinary procedures as well as grooming and hygienic activities while taking care of animal welfare.

Applying positive reinforcement techniques is only possible with appropriate selection of motivators, which requires the knowledge of a given species' biology. Positive reinforcement of desired behaviour in animals positively affects training efficiency [2,9].

Unfortunately, because trainers need to assess the reaction to de-motivating stimulus they often introduce aversive element, i.e: punishment [2,8].

In any case, monitoring the parameters determining animal welfare cannot be neglected. The basis for preparation of training program of each animal species in specific living conditions requires specialist knowledge in the field of biology, in particular of instinctive animal behaviour in its natural habitat [10,11].

Meerkat (*Suricata suricatta*) was chosen to be the subject of study for modelling behavioural patterns with the use of positive reinforcement method.

Meerkat is a herd animal, living in family groups merging into larger colonies (clans) of up to 30 members. The colony is inhabited by maximum 3 family groups. Each family group in natural habitat includes breeding couple and their offspring [12], whereas in captivity breeding in the zoo this structure may not always be maintained, although animals are kept in a herd. Only the alfa couple breeds and the offspring is raised by the entire clan and also other female meerkats in the colony lactate, not only the mother [13]. They can breed every 2 months (in natural habitat usually 3 times a year) giving birth to a litter of 2-5 pups, after a gestation period of 70-77 days. They reach sexual maturity when they are 1 year old.

Within their own clan meerkats are exceptionally friendly to one another, older individuals share their food with the younger ones, they look after them and play with them, while serious battles occur between different colonies [14]. While foraging for food, especially further away from the burrow, one of the meerkats plays the role of a sentry, watching over the colony and warning the members of the herd against the predators with a distinctive bark [15]. More then 10 various vocal signals produced by meerkats have been defined so far, females being observed to be more diversified in their sounds repertoire. Meerkats are active during the day, they leave their burrows on sunny days, while on rainy or cloudy days they stay inside [16]. They usually forage for food in the vicinity of their burrowss, turning over stones and rocks or digging in the sand with their long, hard claws. Meerkats belong to the carnivore order (*Carnivora*) and insects are their staple diet in natural habitat although they also feed on scorpions, millipeds and centipeds, small vertebrates such as lizards, snakes, birds, rodents, eggs and plants. In the zoo they are fed with meat, dried cat food, mealworm maggots, crickets, earthworms and fruit. Their life expectancy is 10 years in the wild and up to 12-15 years in captivity.

This species inhabits Southern Africa (Mozambique, Botswana, Zimbabwe), occupying sandy plains, savannahs and meadows.

The aim of this paper was to develop protocols of procedure which would result in obtaining desired reactions in animals, namely the desired behaviour of meerkats kept in Bydgoszcz

Material and methods

Behavioural training, which was intended to result in modelling 2 behavioural patterns was conducted in group of 4 individuals - 3 females and 1 male (aged 4-8 years old) of meerkats making up one herd.

The herd of meerkats kept in Bydgoszcz Zoo lived in a indoor enclosure located in a concrete building with access to the outdoor enclosure Fig.1. The floor of the enclosure was covered with bedding of sawdust, a shelter was separated, imitating the nest, the box was equipped with a heat lamp, litter tray filled with sand, bowls for water and food and numerous accessories: boxes, tree logs and branches. Meerkats had limited time of using the outdoor area of the enclosure (it was closed at night or due to bad weather conditions) with small stones, branches and underground system of tubes mimicking the tunnels.

Meerkats' diet was balanced and contained food and food supplements adequate for their physiological needs.

Modelling of behavioural patterns was carried out only with the positive reinforcement technique (without punishments). At the outset the desired behavioural reactions were defined. In the experiment in question the effect of behavioural pattern modelling for animals was to voluntarily (without the need of capturing the animal) stay motionless, which would make it possible to read their identification chips in contact-free way and to weigh them. In case of the latter the acceptance of contact stimulus was modelled with a view to familiarising the animal with the caring and veterinary procedures (e.g: injection of medicine). Based on instinctive behaviour the motivator which was recognized and introduced in the experiment was adding to the enclosure such elements as: box filled with plastic balls and a platform with scales on which extra food was placed. Because of the strong hunting instinct in theses animals they were fed with live insects. Daily technological cycle at the zoo was pointed to as de-motivator - animals unwillingly took part in training knowing that food will be given to them at a specific time. Also the undesired individual reactions and strong hierarchy in the herd were recognized as de-motivators, because the social structure of the herd obstructs the training of individual animals and becomes a natural de-motivator.

The experiment was incorporated in animals' routine schedule so as not to disturb their welfare and to be in line with the zookeeping works.

The experiment lasted 4 months, modelling of behavioural patterns according to the developed scheme of procedure was repeated 2 or 3 times a week.

Results

Based on the conducted observations and animals' reactions to stimuli the author's own schemes of modelling selected types of behaviour were developed for meerkats kept in captivity breeding in the zoo.

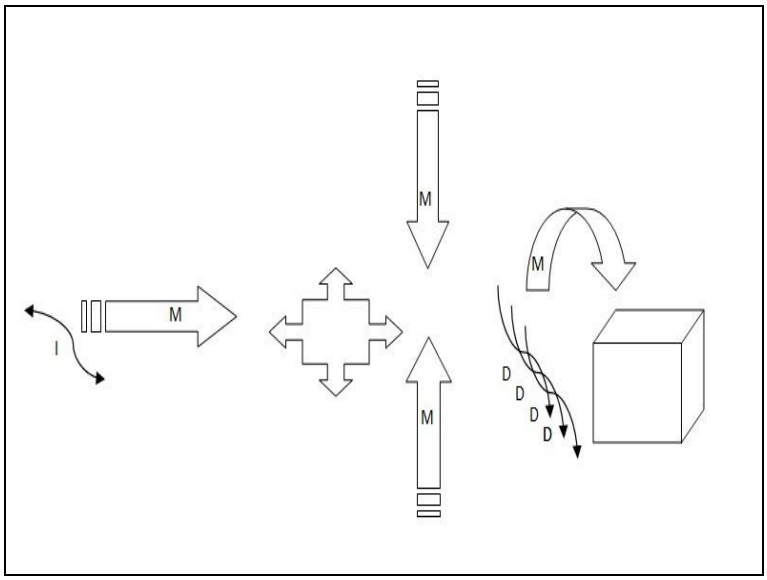

Figure 1. Scheme of modelling desired behaviour patterns in animals
I - Initiation, M - Motivator, D - de-motivator

Behavioural pattern modelling activity was divided into 4 stages leading to the obtainment of desired behaviour:

- initiation of desired reactions
- positive reinforcement of reaction (introduction of motivator)
- subduing undesired reactions in the presence of de-motivator (in case of the experiment - lack of motivator)
- consolidating the desired reactions by prolonging the exposure to the stimulus in the presence of a motivator.

Behaviour-initiating stimulus was the appearance of trainer in the enclosure where animals were kept, his preparation of the experiment area, bringing in the extra elements and rewards for animals. Next the animals familiarised themselves with the extra elements (equipment) which were brought into their surroundings e.g: scales and platform. At this stage reinforcement was introduced - motivators in the form of rewarding the animal with food each time it came in contact with the new equipment.

Then animals start to display transitive behaviour which is especially manifested in social structures. At this stage it is necessary to reduce or eliminate the occurrence of distracting stimuli de-motivators, which is extremely difficult when the number of animals is quite large and they form a herd. Through enhancing the stimuli triggering the desired behaviour we stimulate the conditioning of desired reactions (Fig. 1.).

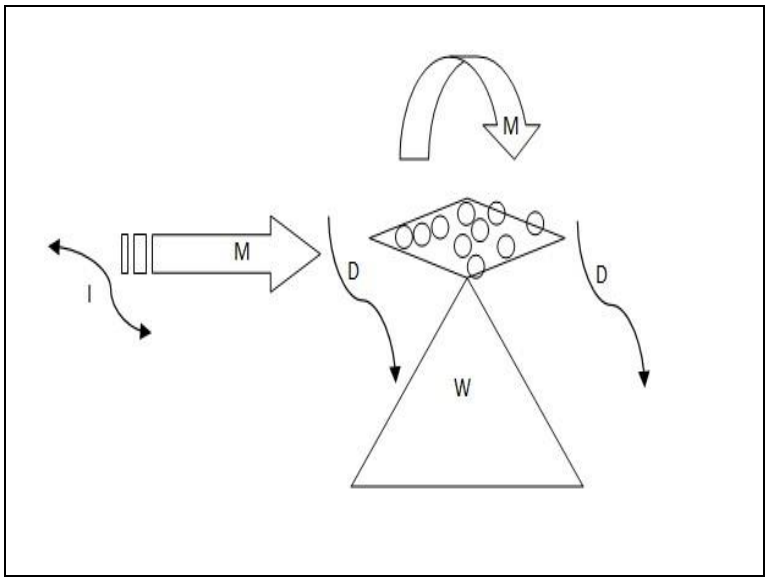

Figure 2a. Scheme of modelling desired behaviour patterns (weighing, reading identification chips) for meerkats
I - Initiation, M - Motivator, D- de-motivator, W - scales+platform with balls

Initiation of behaviour changes in meerkats is marked with the trainer's entrance into the enclosure and bringing in the wooden platform and scales and arranging them in appropriate areas (Fig. 2a.). Placing on the training platform items of food which the animals find attractive (some live maggots) encourages the animals to take action. Hunting instinct, which is very strong in meerkats, triggers a series of reactions and those desired ones are reinforced by the trainer by rewarding the animal with an extra treat. Filling the platform with lightweight, small balls, into which animals dived and looked for treats - maggots was a method of mimicking the natural habitat in the enclosure and allowing meerkats to hunt and forage for food (Fig. 2a.).

The size of the herd was making it difficult to make observations during the experiment which can be considered to be a de-motivator in the course of training, as well as the action of rewarding only those individuals who performed the task. Daily routines of zookeepers and other zoo staff which brought about certain acoustic and olfactory stimuli were also distracting factors for the animals during the training. Additionally, the daily cycle of cleaning and feeding which results in increased intensity of activity (e.g: during feeding hours) or its lack (rest after feeding) was a factor that obstructed conducting the training.

The experiment was considered to be successful when the animal stayed on the platform for about 30 seconds which enabled the trainers to weigh the animal or to read the numbers on its identification chip (Fig. 2a.).

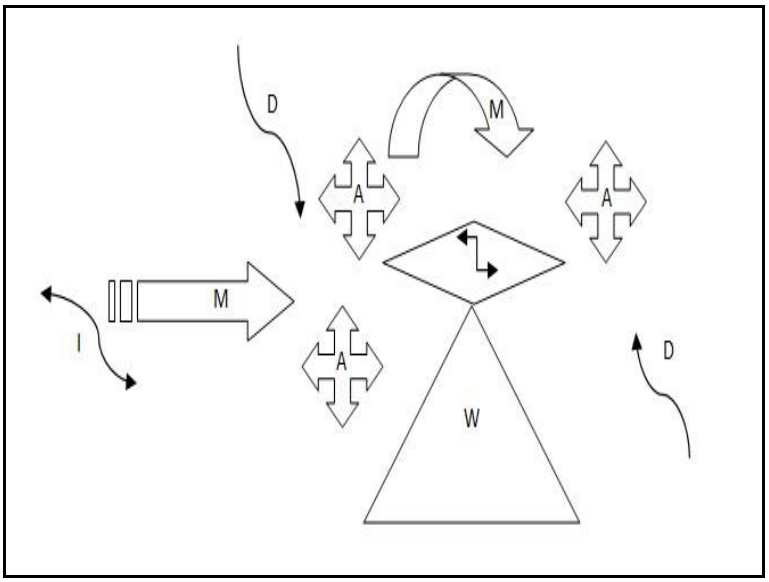

Figure 2b. Scheme of modelling desired behaviour patterns (injection) for meerkats
I - Initiation, M - Motivator, D- de-motivator, W - scales+platform with balls, A- different animals

In case of preparing the meerkats for the acceptance of contact stimulus the initiation and acclimatisation stage were similar to the training described above, i.e: the trainer entering the enclosure with the items used in the course of the experiment. The applied pattern and the choice of motivators made it possible to introduce the contact stimulus. In the initial phase of the experiment only the individuals holding a high position in the hierarchy of the herd dared to stay on the training platform for up to 30 seconds and put up with being touched with tweezers in the presence of motivator (Fig. 2b.). For the individuals on the lower level of social hierarchy in the herd the additional motivator was the pattern of behaviour displayed by the dominant individuals (mirror reaction was the motivator in this case).

Discussion

According to Shapiro [9] the main determinant of training efficiency with the use of positive reinforcement is the timespan between the moment of initiation of behaviour and manifesting by the animal the series of reactions constituting the desired behaviour. In the experiment the first reaction of meerkats influenced by motivators occurred already during the first training session. What proved to be difficult was isolating the individuals in terms of reaction to stimulus, not the act of initiating the desired behaviour itself. In social structure there is a strong hierarchy and each member of the herd plays a specific role [15,17]. Any destabilising action towards that structure, even the one evoked by positive reinforcement results in conflict within the group. Visible interactions among meerkats are modelled by all environmental factors and force the animal to adopt individual behavioural strategies [18,19,20]. Even when rewarded, isolation from the herd causes stress, particularly in the species such as meerkats, whose lives always revolve around the family guaranteeing them safety [21,22].

In the experiment there was a diversified degree of individual reactivity with the use of de-motivator, i.e: lack of reward when the animal was too agitated. In the observed group the dominant role was adopted by the male who had already displayed desired behaviour at the moment of the introduction of extra elements in the surroundings before the presence of motivator, i.e: he entered

the training platform and stayed there not letting other members of the herd access the platform while he was waiting for food, instead entering the platform after being encouraged by food placed on it. The attempt was made at decreasing the degree of dominant male's reactivity while increasing simultaneously the degree of agitation in other herd members. Manifesting position in the social structure becomes most conspicuous in the presence of food since the competition for food constitutes the main cost of living in a group [21,22].

The choice of live food as motivator brought about the desired result in case of meerkat training: it realised their need to hunt as well as the need to forage for food. Because the animals kept in the zoo should not be accustomed to direct contact with humans, i.e: stroking or handing out food the application of training tools such as platform or tweezers seems to be justified. Moreover, if the elements and tools stay the same the scheme of procedure (behavioural pattern) is maintained and repeated, the animal will display desired behaviour regardless of the person initiating the reaction, and, consequently, the development of the effective scheme in case of the necessity of its practical application during caring and veterinary procedures does not require the presence of trainer or zookeeper but merely the re-enactment of the behavioural pattern.

Behavioural trainings may be used in order to minimize stress in a situation when animals are moved to a new enclosure, in which the prompt introduction of behavioural pattern makes it possible to shorten the acclimatisation period and to introduce new elements in the environment. Animals are willing to cooperate and expect positive reinforcement while not being afraid of unknown stimuli.

Conducting systematic trainings is a basic underlying principle of pattern modelling and only consistent execution of protocol without exceptions allows us to expect the occurrence of desired behaviour [4]. The introduction of positive reinforcement techniques in animals leads to decreased level of stress hormone (cortisol) in the blood, lower miscarriage rates and fewer cases of stress-related diseases as well as to increased physical resistance to manipulation and fear [7]. In view of the above it is easier and safer both for the person carrying out tests and for the animal itself to perform caring and veterinary procedures as well as tasks related to animal physiotherapy and breeding [5,6].

Conclusions:

1. Behavioural pattern modelling based on a cycle of animals' desired reactions to an indicated stimulus must be proceeded by defining the motivators and de-motivators specific for a given species and a given individual while taking into consideration all the conditions in which animals are kept.
2. Behavioural pattern modelling of animals living in zoos positively affects their welfare, reducing stress related to breeding procedures, caring and veterinary activities.
3. Behavioural training conducted among meerkats proved to be difficult due to strong social structure and herd-induced behaviour of those animals. Animals in a herd play defined roles and working with a selected individual required additional provision of stimuli.

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MORPHOMETRICS IN MOVEMENT BIOMECHANICS OF EGYPTIAN FRUIT BAT (*ROUSETTUS AEGYPTIACUS*)

Monika Lik*, Tamara Samsonowicz, Ewelina Zielińska
Rada Naukowo-Dydaktyczna, Leśny Park Kultury i Wypoczynku, Myślęcinek, spółka z.o.o.,
ul. Gdańska 173-175, 85-674 Bydgoszcz
*corresponding author: lik.m@tlen.pl

Abstract:

The aim of this paper is the attempt to assess the influence of the morphological structure on the movement efficiency in Egyptian fruit bat. This paper presents the results of morphometrics of a flock of 30 adult individuals of Egyptian fruit bat kept in Bydgoszcz zoo. The animals were kept in a caged enclosure inside the building, isolated from weather conditions. In the flock there was a majority of females, 24 individuals, and only 6 males. Quite significant diversity was observed among male and female individuals in terms of described morphometric parameters (bodyweight, body length and body width or wingspan) which was further reflected in pyrometry results and flight quality. The results presented in this paper are to be regarded as preliminary research, the systematic continuation of which will enable us to create a database of standard values for morphometric parameters of Egyptian fruit bat kept in captivity breeding (in the zoo).

Keywords:

motphometrics, Egyptian fruit bat, movement efficiency

Introduction

Morphometrics is, in other words, a parametric designation (measurement) of the elements of animal's body's shapes and sizes.

Although morphometrics is applied in all scientific disciplines, it plays the most important role in zoology, where detailed and accurate description of animal's morphology is the basis for finding the connections (correlations) between the sizes of various body parts, organs and structures and their functioning [1].

Thanks to undeniable evolutionary advantage which bats acquired as the only mammals, namely the ability of powered flight, their wings are usually measured for morphometric purposes (e.g: [1, 2,3]). Flight-assisting membrane in bats (patagium) stretches between elongated digits and the side of the body to the hindlimbs. Sometimes flight-assisting membrane appears also between hindlimbs.

On the surface of the patagium the following parts can be distinguished: membrane anterior to the wing (protopatagium), interdigital membrane (chiropatagium) - between the third and the fourth digit and between the fourth and the fifth digit, wing membrane (plagiopatagium) with the largest surface and stretching between the fifth digit and the side of the body and tail membrane (uropatagium).

Bats' manner of flying is characterised by very high manoeuvrability thanks to the possibility of decreasing the surface of patagium (partial folding of wings results in bulging of patagium) which in turn reduces the speed but makes it easier to perform turns. They also, unlike birds, have the ability to move their wings alternately. Frugivorous species usually fly slower than insectivores and their wings are relatively wide and rather short [4,5].

Incredible manoeuvrability is the result of specific wing structure, equipped with over 20 joints which makes them very flexible, every digit moves independently and membrane stretched over thin bones may expand in order to catch the air currents and create lift force. It gives bats control over three-dimensional shape of their wings, which is created during the flight [4,6], however birds surpass bats in terms of flight effectiveness. Bats' wings also serve thermoregulation purpose. During the flight 80% of energy is released as heat [7], which is radiated by wings, preventing hypothermia. Balance between thermogenesis and loss of heat is particularly important for bats who spent a longer time flying or those living in tropical and sub-tropical zones [7].

The exotic species - Egyptian fruit bat, which is kept in captivity breeding in Bydgoszcz zoo, was chosen to be the subject of the experiment.

Egyptian fruit bat (Rousettus aegyptiacus) belongs to suborder of Megachiroptera, consisting of only one family - fruit-eating bats.

Bats belonging to this family are rather large compared to domestic bat species (representing Microchiroptera suborder), the wingspan of Egyptian fruit bat is approx. 60 cm, body length is up to 20 cm and body weight is 80-170 grams, while females are larger than males. Both males and females are of similar colour - typically dark brown or grey with lighter belly and darker back. Wing membranes are dark brown and covered with fur up to mid-forearm. Only the first and the fifth wing digit has a claw, and hindlimbs have 5 clawed digits each [8,9].

Due to the fact that this species is active at night their eyes are large and adapted to night vision [10]. They forage for food mainly at night and return to their hiding places before sunrise. Among Megachirotera suborder members only Rousettus genus uses echolocation mainly because of the fact of foraging for food at night; bats produce and transmit sounds - tongue smacks - for spatial orientation purposes [11,12]. A single tongue smack made by Egyptian fruit bat lasts about 0,6-1 ms, and has the frequency of 12-70 kH [13].

During breeding season females separate from males, forming a maternity colony, while males are grouped in a bachelor colony.

After a gestation period of about 4 months females give birth usually to a single young bat, less often twins, who stay with their mother until they reach the age of 9 months [8,10].

Egyptian fruit bat inhabits the territory between sub-Saharan Africa and North Africa, Middle East as far as Turkey, South-East Asia and Cyprus, in form of numerous sub-species [14,15,16].

It is a long-lived species - in their natural habitat the average life expectancy is approx. 10 years, in captivity they can live for up to 25 years [10].

In Africa the populations of Egyptian fruit bat are numerous and form huge colonies of up to 50 thousand members, therefore in certain regions they are hunted by indigenous peoples.

Egyptian fruit bat is rarely encountered in South-East Asia, with their flocks of up to 50-500 members [15,17].

Bats can migrate over considerable distances, contributing to the dissemination of zoonoses, which in case of Egyptian fruit bat is a dangerous Marburg virus posing a deadly threat for humans,

for instance in Kitaka cave located on the border between Kenia and Uganda as many as 5% of Egyptian fruit bats, i.e: 5 thousand of bats carry the virus [18].

This species is regarded by IUCN to have the lowest conservation status (LC) [19,20].

The aim of this paper is the attempt to assess the influence of the morphological structure on the movement efficiency in Egyptian fruit bat.

Material and methods

This paper presents the results of morphometrics of a flock of 30 adult individuals of Egyptian fruit bat kept in Bydgoszcz zoo. The animals were kept in a caged enclosure inside the building, isolated from weather conditions. In the flock there was a majority of females, 24 individuals, and only 6 males.

In each gender group the same measurements were conducted. The animals were weighed with the use of PESOLA scales of maximum load up to 300 grams (Fig. 1.).

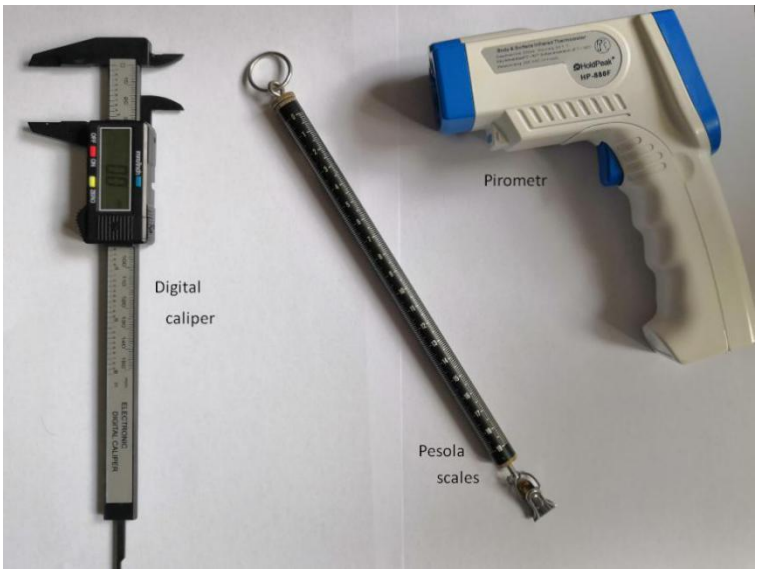

Figure 1. Measurement tools used in research

In this article bodyweight was marked with **mc** symbol.

The length of particular body parts was measured with the use of digital calliper (Fig. 1.). Morphometrics consisted of the following measurements:

- body length, which was defined as the distance from the front edge of the nose to the base of the tail - in the text marked as **d**,
- body width - the distance between shoulder joints - in the text marked as **s**,
- length of arm with forearm (from arm joint to the wrist) - in the text marked as **r1** value,
- length from wrist along the third digit to the last phalangeal joint of the third digit - in the text marked as **r2** value,
- length of the third phalange of the third digit - in the text marked as **r3** value.

In order to obtain the result defining wingspan (marked in the text as **RS**) the following formula was applied:

$$RS=(r1+r2+r3)x2 +s \quad (1)$$

Additionally, body temperature was measured twice with pyrometer (Figure 1.) - once right after the animals were captured **(t_u)**, another measurement was taken after the effort of a short-term free flight **(t_a)**.

Statistical characteristics was to designate measurements of location and changeability within the range of researched features taking into account the animals' gender. Dispersion of values in researched characteristics for each gender was also defined. Statistical characteristics were performed using Statistica 12PL StartSoft package. The attempt was made to find correlations (Spearman's correlation) between researched morphometric parameters and the results of pyrometry, however no statistically significant correlations were detected, therefore these results were not described in the further parts of this paper.

Results and discussion

The bats described in the test were mature individuals whose somatic growth had ended, thus the conducted morphometry is a characteristic of the whole flock and may be used to create a database for the species kept in the zoo. The average bodyweight of Egyptian fruit bat was 169 grams and was in the upper range of standard for this species, [10], which, defined at bodyweight spread, ranges from minimum value of 115 grams to maximum value of 210 grams in the largest male (Tab. 1.).

Table 1. Statistical characteristics of research group

parameter	$x_{śr}$	min	max
mc	169,47	115,00	210,00
D	13,38	11,00	15,02
S	4,74	3,61	6,68
r1	10,64	7,62	13,64
r2	9,67	8,30	10,82
r3	5,10	4,05	6,11
t_u	38,06	28,00	40,50
t_a	38,10	36,00	39,80

Source: own calculations

Referring to available publications on the subject [10,14] it was observed that body length of researched animals is lower than the average for Egyptian fruit bat, but it must be stressed that body length measured in this research was defined as the distance from the edge of the nose to the base of the tail, excluding the tail itself. The average body length was therefore over 13 cm (11 cm minimum to 15 cm maximum), with the average width of about 5 cm (from 4,7 to 6,6 cm) - Tab. 1.

The greatest discrepancies in morphological structure were noticed in case of parameter r1, where the difference between the extreme values was almost 6cm. Morphometry results of the 3rd digit were not as diversified and their maximum amplitude was over 2 cm (Tab. 1.).

The range of surface pyrometry measured in Egyptian fruit bats was from 38℃ to 40,5℃ when animals were motionless, whereas after the flight it varied from 38℃ to 39,8℃.

Obtained results, particularly for surface thermal range measured in motionless stage (in a grip) were higher than body temperature recorded by Barclay et al. [21]. The aforementioned authors examined bats living in the wild, which inhabited a cold cave in Southern Africa (where temperature was 10℃ or lower) and the measurements were not taken while bats were captured in a

grip - an activity which generates stress in animals in their natural habitat. The temperature ranges recorded for Egyptian fruit bats were from 37,7℃ after returning from foraging for food flight to 35,5℃ during the period of rest [21]. Korine and Arad [22] state that the body temperature normal for this species is within the range of 31-36℃, assuming that the body is optimally hydrated. They carried out their research in Israel, in a relatively hot climate and came to a conclusion that Egyptian fruit bats living there adapted to maintain adequate thermoregulation even in the event of water shortages [22].

Comparing bodyweight of males and females it was noticed that males were heavier, which is a standard in case of Egyptian fruit bats [10]. Bodyweight of every male was above 150 grams, and the average weight of male bats was about 180 grams (Fig. 2.).

Females were on average about 15 grams lighter than males and weight of female bats was more diversified: it ranged from 115 grams to 215 grams (Fig. 2.).

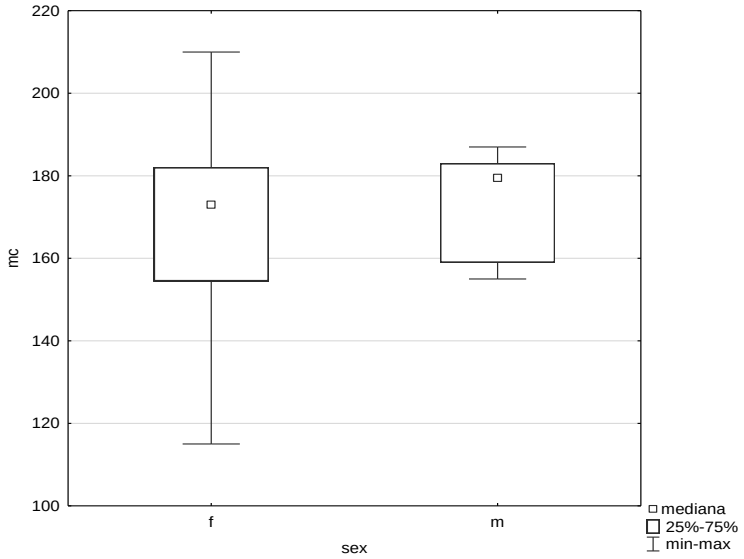

Figure 2. Dispersion of obtained bodyweight values - mc (in grams) of females (f) and males (m) of Egyptian fruit bat
Source: own calculations

A similar tendency was observed in case of the second morphometric feature - body length. The difference between body length in female and male bats was on average slightly over 0,5 cm (Fig. 3.). Females were characterized by greater diversity in terms of body length, from very small individuals 11cm to 15cm (Fig. 3.).

In comparison with the results obtained by other authors, e.g:. [10] the data concerning body weight obtained in this paper are lower, but this resulted from difficulty in measuring the tail, the measurement of which was eventually left out. The mean value of body width in males and females was similar - the difference in favour of males was less than 0,5 cm (Fig. 4.).

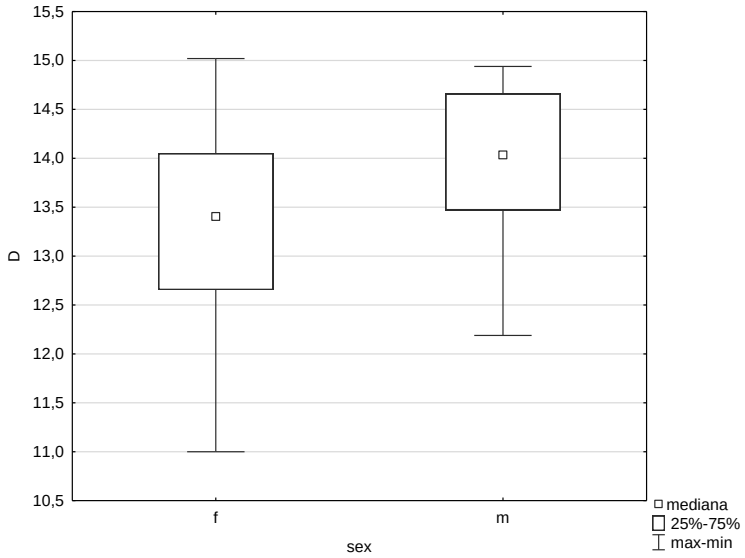

Figure 3. Dispersion of obtained body length values - D (the distance from the front edge of the nose to the base of the tail) of females (f) and males (m) of Egyptian fruit bat (in cm)
Source: own calculations

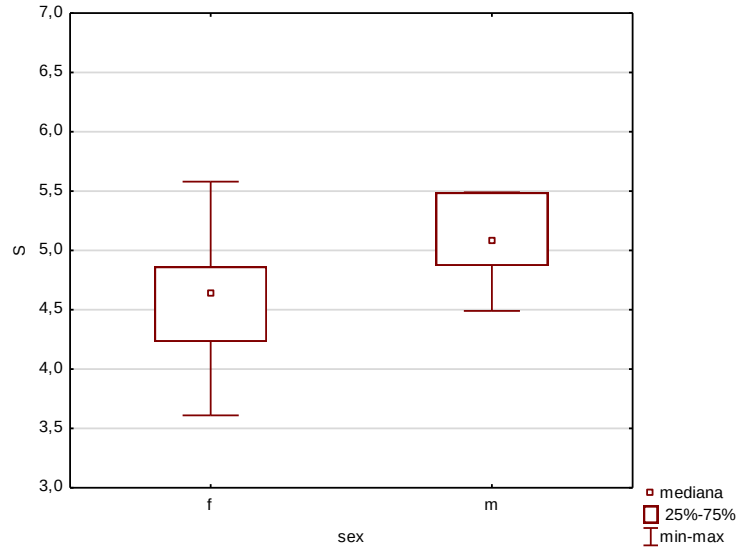

Figure 4. Dispersion of obtained body width values - S (the distance between shoulder joints) of females (f) and males (m) of Egyptian fruit bat (in cm)

Source: own calculations

Increase of value in all the described parameters each time affects bats' movement efficiency, requiring a higher energy expenditure, especially during flight. The basic positive feature affecting flight is the supporting structure of wings depending on the morphological components. 3 wing component parameters were directly characterized - r1-r3, and their total sum makes up the wing length.

The occurrence of sexual dimorphism was confirmed - female bats had shorter wings (Fig. 5-7).

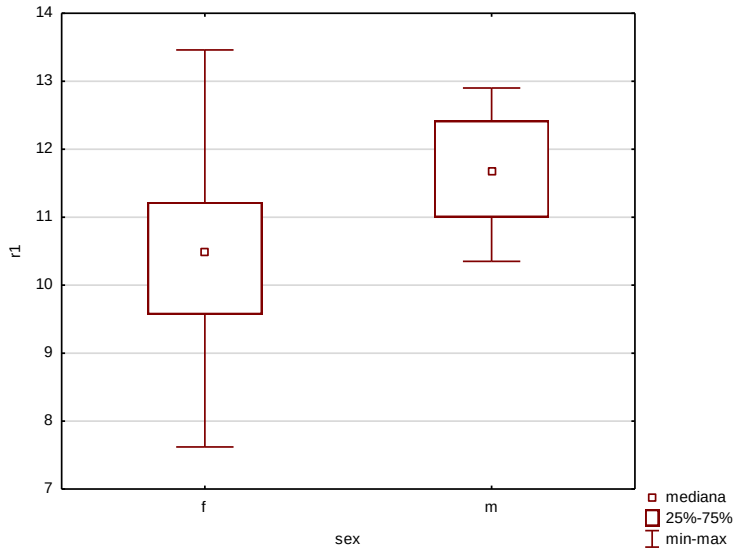

Figure 5. Dispersion of obtained r1 length values (the distance from shoulder joint to the wrist) of females (f) and males (m) of Egyptian fruit bat (in cm)
Source: own calculations

The length of section from arm to the wrist was in females on average 10,5 cm whereas for males it was 11,5 cm (Fig. 5.), from the wrist to the end of the second phalange of the third digit 9,5 cm in females, in males almost 10 cm (Fig. 6.), and the length of the third phalange of the third digit respectively 5,1cm and 5,3 cm (Fig. 7.). Within 3 wing parameters in females there was greater diversification in each measurement (Fig. 5-7.).

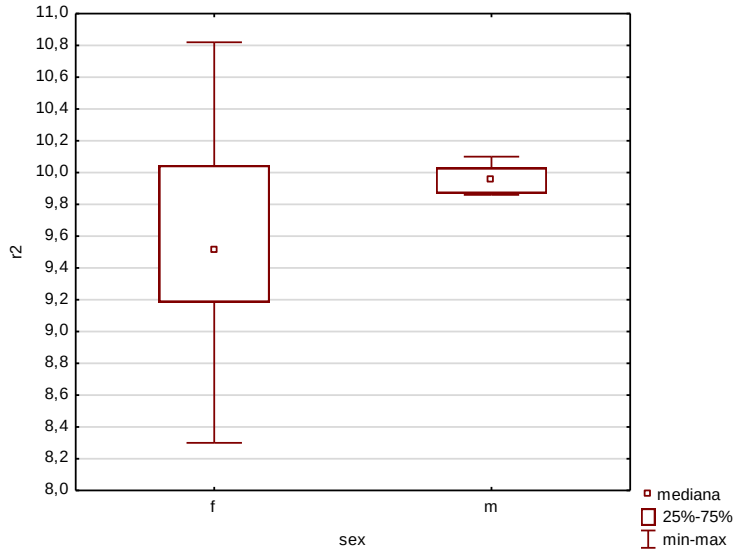

Figure 6. Dispersion of obtained r2 length values (the distance from the wrist to the third phalangeal joint of the third digit of the wing) of females (f) and males (m) of Egyptian fruit bat (in cm)
Source: own calculations

Bats' bodies generate less lift force than birds' bodies which results in their uneven distribution of force and lower efficiency. Birds fly faster, migrate more frequently and further than bats [6,23,24]. Variation rates of bat flight were tested in aerodynamic tunnels and compared with results achieved by birds [25].

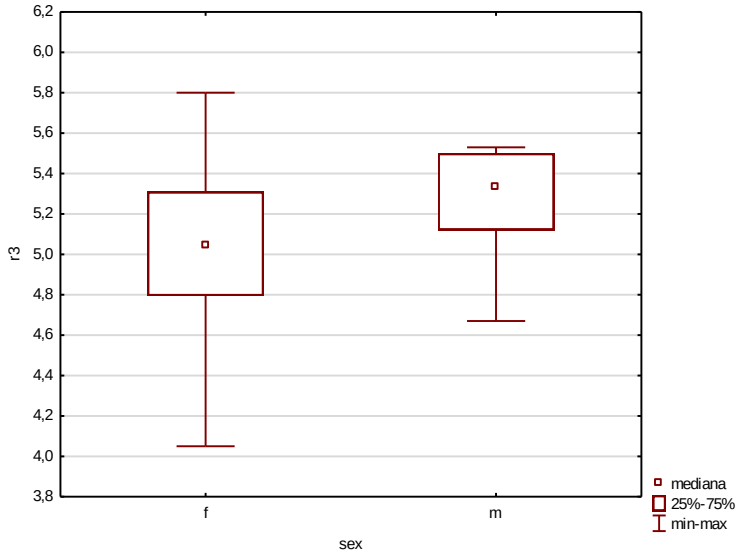

Figure 7. Dispersion of obtained r3 length values (length of the third phalange of the third digit of the wing) of females (f) and males (m) of Egyptian fruit bat (in cm)
Source: own calculations

Table 2. Wingspan of females (f) and males (m) of Egyptian fruit bat (in cm)

Lp.	**Sex**	**Wingspan (cm)**	**Lp.**	**sex**	**Wingspan (cm)**
1	f	47,44	13	f	57,36
2	f	55,46	14	f	53,70
3	f	51,17	15	f	51,94
4	f	52,92	16	f	48,84
5	f	53,73	17	f	55,95
6	f	59,12	18	f	61,58
7	f	49,18	19	f	58,87
8	f	56,46	20	f	60,53
9	f	49,13	21	f	52,78
10	f	54,36	22	f	58,94
11	f	57,55	23	f	57,40
12	f	55,33	24	f	52,89
Average (f)			**54,69**		
1	m	57,17	4	m	59,11
2	m	58,23	5	m	57,75
3	m	60,38	6	m	60,49
Average (m)			**58,85**		

Source: own calculations

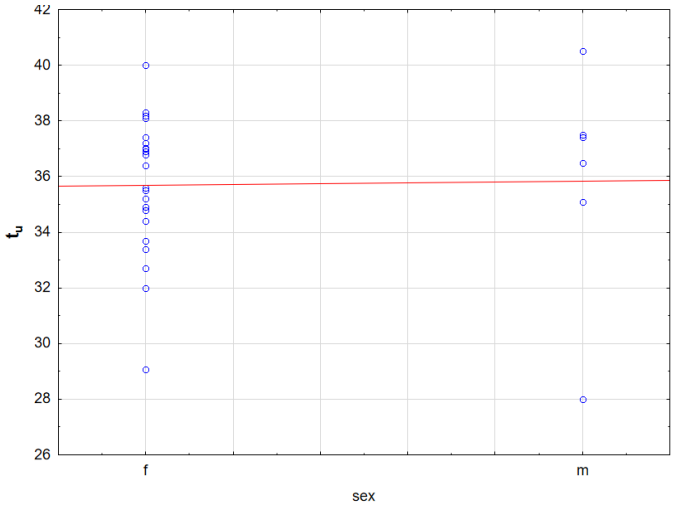

Figure 8. Dispersion of obtained body temperature values during the period of immobilising -t_u females (f) and males (m) of Egyptian fruit bat (in ℃)
Source: own calculations

The pace of bats' metabolism is fast as the energy expenditure for inefficient flight, fast turns (especially in insectivores) results in higher demand for energy in order to maintain all vital functions, including stable body temperature. The quality of flight is positively affected by wingspan described for the researched group in Tab. 2..

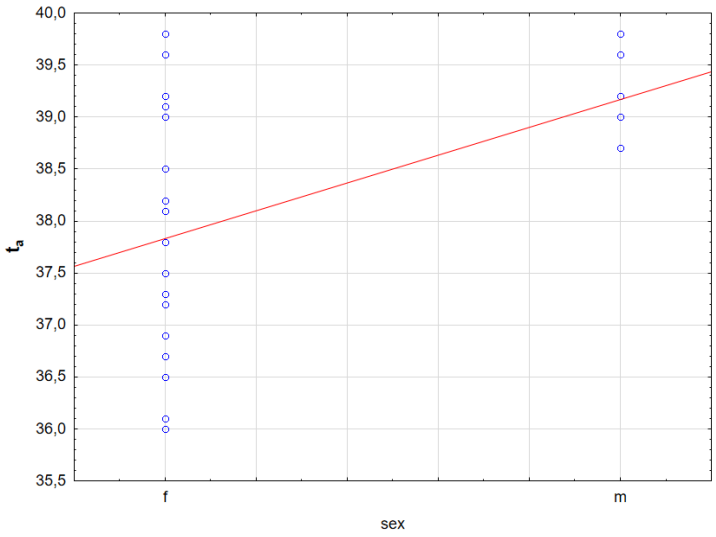

Figure 9. Dispersion of obtained body temperature values after a free flight t_a in females (f) and males (m) of Egyptian fruit bat (in ℃)
Source: own calculations

Unfortunately, at this (preliminary) stage of research the measurements were taken only once and it was not possible to establish direct correlation between the amplitude of surface thermal range and wingspan of Egyptian fruit bats. Nevertheless, most likely because of sexual dimorphism, the wider wingspan was observed in male bats where it was on average 58,8 cm and was 4 cm more than in female bats.

This did not affect the differences in average surface thermal ranges recorded for the animals during rest period in terms of gender (Fig. 8.) but at the stage of free flight the individuals with wider wingspan (males) displayed higher thermal activity than females, which, in researchers' opinion, is connected with higher bodyweight and flight quality (Fig. 9.)

Flight of male bats is more dynamic and is characterized by higher manoeuvrability compared to females, which was already noticeable in the preliminary research.

In order to make direct link between the above mentioned correlation and to make its statistical description the research will be continued.

Conclusions:

1. Quite significant diversity was observed among male and female individuals in terms of described morphometric parameters (bodyweight, body length and body width or wingspan) which was further reflected in pyrometry results and flight quality.
2. Stress connected with forced motionlessness (grip) and limiting the possibility of movement was comparable for both genders and the average values of surface thermal range remained on the same level regardless of gender.
3. Wingspan as the element of morphogical structure directly influenced the increase of the thermal activity and, consequently, indirectly affected metabolic activity in Egyptian fruit bat's active flight, which was in the research particularly visible in the assessment of the aforementioned parameters in male bats.
4. The results presented in this paper are to be regarded as preliminary research, the systematic continuation of which will enable us to create a database of standard values for morphometric parameters of Egyptian fruit bat kept in captivity breeding (in the zoo).

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THERAPY FOR VICTIMS OF POST-TRAUMATIC STRESS

Szymon Peterman
Uniwersytet Opolski
szymon_peterman@yahoo.ie

Abstract:

Post-traumatic stress disorder is classified as an anxiety disorder, an injury that can arise as a result of traumatic psychiatric and physical survival. Strong stress is associated with life experience in which life and human health have been endangered or a person has witnessed dramatic events. Situations that can cause this stress include, among others: serious communication accident, being injured as a result of crime (assault, rape) or being a victim of domestic violence. Post-traumatic stress disorder is not a disease, but what specialists call the "wound of the mind". It depends on us whether the negative symptoms will accompany us, disappear, soften or be minimized. People with post-traumatic stress disorder can not get rid of the brutal situation of the past and remain in it. The present and the thought of the future are being replaced by negative memories in which they are stuck.

Keywords:

anxiety disorder, stress, negative memories

Posttraumatic stress disorder PTSD - characteristics, personality and symptoms of stress

Post-traumatic stress disorder is usually referred to as a mental disorder or injury that is a consequence of dramatic events in which the person participated directly (victim, injured) or indirect (witness), such as a robbery using a dangerous tool, a transport accident or domestic violence. Terrifying thoughts associated with these events are transformed into mental suffering associated with the constantly recurring memories of a traumatic experience.

Although the term "post-traumatic stress disorder" itself appeared in the early eighties, such symptoms, which are described in patients with this diagnosis, have already been the subject of psychiatric and psychological analysis. I assume that the "war neurosis" described by psychoanalysts, which was diagnosed in many soldiers after the First World War, corresponds symptomically to today's PTSD, especially among patients who were veterans of the Vietnam War. Similar analogies can be noticed in the case of research on people who survived a stay in a concentration camp. It is worth noting that it was in Europe for the first time that researchers dealing with mental disorders pointed out that strong, traumatic experiences may to some extent disturb the mental functioning of a human being. Continuing to interest in this issue, members of the American Psychiatric Association over 20 years ago noticed among the aforementioned veterans of the Vietnam War, permanent and profound changes in mental and psychophysical functioning. During this period, research focused on the type of traumatic experiences and the resulting

psychological consequences, but not related to war. These symptoms were related to violence, violence against children and women [1].

One of the most important conceptual dilemmas related to post-traumatic stress is the explanation of the key role of the terrifying experience as determinants of mental disorders and the comparison of this role with the influence of personality. This dilemma appeared already in the nineteenth century, when the dominant views on the etiology of mental disorders oscillated around two poles: environmental perspective and genetic perspective. Commonly cited concepts, such as constitutional predisposition, were intended to explain the causes of mental disorders. The views prevailing during the First World War are expressed by the opinion of the chief military psychiatrist of the United States, who stated: "Considering the causes of these diseases, one can not omit predisposition; it is widely believed that this factor is clearly enough to justify rejecting a recruit in most cases. " The above-mentioned opinion testifies to the primary role attributed to the personality considered the primary cause of war neurosis. Such views provided strong arguments justifying the stigmatization of people who broke down during the fight or suffered mental disorders in the aftermath [2]. In Poland, for the first time, the diagnosis of PTSD has become widely recognized during the military operations of Polish contingents in Iraq. At that time, public media, in particular the press, TV and radio programs in 2004-2005 alarmed public opinion about the significant incidence of PTSD in Polish soldiers of the peace mission. Some sounded very scary and ominous, stating that almost every third soldier returning to the country has symptoms of PTSD with its health, family and social consequences [3].

Situations that cause stress in people are an inseparable part of life. Every day we struggle with some more or less stressful situations. The non-essential ones that have not touched us too much are thrown out of our memory. They have not exerted such a strong influence on us that they are constantly being remembered - they are replaced by others, new ones. The moderate tension that causes stress in each of us seems to be a natural factor. It is what makes us able to find ourselves in a difficult situation, make a quick decision and face danger in an appropriate way. Not always stress occurs as a negative factor. In certain people who find themselves in a difficult, critical life situation, without seeing the possibility of resigning from the situation, stress is the motivator to act, to find new ways and solutions. Often after the trauma in which they were, these people discover themselves again, noticing their potential and belief in their own strengths and abilities. They start to believe in themselves. Stressful situation is no longer something impossible to overcome, but it becomes a motivator to find accurate and rational solutions. Such optimistic situations depend, however, on many factors such as: the personality of a person or the effects of a traumatic event that a person has suffered. Sometimes, however, the consequences of severe stress turn into anxiety disorders called post-traumatic stress disorder and are characterized by the presence of various types of symptoms.

As typical, symptoms are described, including episodes of repeated re-experiencing trauma in intrusive memories (reminiscences) or in dreams. These symptoms occur in the context of a persistent feeling of numbness and emotional dullness, isolation from other people, non-reaction to the environment, anhedonia and avoiding actions and situations that might resemble an experienced trauma. There are frequent concerns about memories reminiscent of a patient's trauma leading to their avoidance. Sometimes dramatic, sharp outbursts of fear, panic or aggression can occur, triggered by stimuli that cause a sudden recall or re-experience of a traumatic situation or initial

reaction to this situation. Usually, there is a state of excessive stimulation of the autonomic system with increased wakefulness, increased orienting instinct and insomnia. Symptoms and anxiety often coincide with the above signs and symptoms, and often suicidal thoughts accompany it. An additional factor complicating the image is the abuse of alcohol or medication. The onset of the disorder occurs not immediately after the injury, but after a latent period, which can last up to several months, rarely exceeds half a year. The waveform is heaving, changing. In most cases, the symptoms disappear completely. In a small percentage of people, the disorder may be chronic, long-term, with a transition to permanent personality change. Predisposing factors (including specific personality traits, prior neurotic decompensation) may lower the reaction threshold when this syndrome appears or exacerbate the course of the disorder. However, they are neither necessary nor sufficient to explain its origins. The PTSD symptoms described above can be grouped into four categories:

1. Excessive arousal, a state of constant readiness - feelings as if the danger was to return at any moment; this condition is manifested by an accelerated heart rhythm and elevated arterial pressure, sweating, anxiety, sensitivity or indifference to stimuli, and reacting with disproportionate anxiety and vigilant sleep.
2. Experiencing a catastrophe in dreams and recurring memories - the emotions that accompany them may be equal to those that a person felt at the time of the tragedy.
3. Narrows of perception and behavior - state of numbness, isolation from emotions, partial anesthesia or loss of individual sensations, emotional indifference and passivity.
4. The guilt of the survivor - feeling guilty of those who did not survive, meditation about his helplessness, about the fact that it was possible to help, prevent tragedy, experiencing guilt due to the reflexive rescue of his own life [4].

Therapies and styles in the treatment of post-traumatic stress

Systematic desensitization - a therapeutic technique used in the treatment of anxiety disorders, proposed by the psychiatrist Joseph Wolpe (otherwise systematic desensitization). It is based on teaching the patient to obtain a state of emotional relaxation and entering a state of relaxation in the presence of an object or phenomenon that causes fear. It is based on the obvious assumption that fear and relaxation, being mutually contradictory phenomena, can not coexist - fear is therefore attempted to be eliminated by replacing it with a state of relaxation [5].

The earliest work on the application of behavioral rules to victims of trauma concerned systematic desensitisation. This procedure involves combining the imaginations of a fearful stimulus with relaxation and usually requires the individual to contact such ideas in a hierarchical order. Although two studies on war veterans, in which the effects of systematic desensitization with the functioning of people in control conditions without therapy were compared, demonstrated its effectiveness, it required many sessions, and the change in PTSD was not diagnosed directly. Brom, Kleber and Defares compared the effectiveness of systematic desensitization, hypnotherapy and short-term psychodynamic therapy in a mixed group of victims of injuries with a control group - people on the waiting list for therapy. After an average of 15 therapeutic sessions, subjects under active therapy demonstrated greater improvement than the control group. A group of systematic desensitization showed a slightly greater improvement, but this difference was not statistically

significant. Frank and Stewart tested the effectiveness of systematic desensitization in a series of research on women victims of rape. In these studies, systematic desensitization consisted of imagining traumatic scenarios combined with positive images. Although these authors stated that the therapy led to the reduction of fear and improved the social adaptation of clients, their conclusions were of limited strength, because there was no control group, no PTSD measurement tools were used, and 75% of subjects tested themselves in vivo. In addition, a certain extent of improvement could have been the result of natural adaptation, as some of the participants were subjected to therapy in the acute phase of reaction to injury. In general, although systematic desensitization shows some successful results, methodological failures of the available research make it impossible to draw unambiguous conclusions about its effectiveness. Other therapies such as exposure therapies may take the form of imagery or in vivo. While in vivo exposure requires the individual to be in close contact with real stimuli, the imaginal exposure is based on imagining the events of injury by the individual or recalling their memories. In this type of therapy with regard to PTSD, imaginative exposure was primarily used, due to the need to gain access to traumatic memories. In most protocols of the imaginative exposure, the client is asked to focus on traumatic memories in a way that activates the entire distress associated with these memories. This form of prolonged exposure usually requires focusing on the injury for at least 50 minutes - it is assumed that after this time habituation will begin [6] - a process in which the response to a given stimulus with its repetitiveness ceases with time. Another therapy is so-called cognitive therapy, in which people live wrongly believing that they are prone to suffering harm. The effects of these and other therapies, such as anxiety or combined techniques, have not been clearly identified as positive. Often, the research trials were relatively small or control conditions were lacking. Initial attempts of these therapies, however, have their usefulness. It should be emphasized that the achievement of mental balance is a process staggered over time. Some recover it quickly, others by tedious and hard work on their own personality. In each case, the individual population after the injury is different.

In most stress concepts, it is assumed that at the time of his / her survival, the person attempts to deal with him / her. There is some controversy as to whether these attempts should be described in the terms of the strategy, i.e. ways to combat stress, or in terms of style, which presupposes a certain stability or preference in the application of specific remediation strategies. In the second approach, the style is treated as a personality trait. It can be assumed that there are such strategies or stress control styles that more effectively than others will help the individual adapt to the traumatic event and the stress he or she experiences in connection with this event. People can use different ways to combat stress, which can be grouped into different categories. Accepting as a basis for neurological deliberations the mechanism of post-traumatic stress disorder, an attempt was made to determine what personality traits may promote the formation of nervous hypersensitivity of the limbic system and persistence of this sensitivity for a long time. It can be assumed that the ease of creating sensitivity in the nervous system will depend on its properties. Starting from the classification of the central nervous system types, they will react with a stronger stimulation during experiencing trauma (they will experience a high level of tension) than people with a stable nervous system. The results of the study confirm that the second type of people is more resistant to stress, and their behaviors are less distorted in a highly emotional situation. It was assumed that people with a higher rate of strength of the agitation and inhibition processes will be more resistant to the occurrence of symptoms and the development of post-traumatic stress disorder. Another feature that

can differentiate the duration of tension may be the mobility of nervous processes as a temperamental feature. Mobility manifests itself in the speed of excitation and disappearance of nervous processes. It can be expected that people with higher mobility of nervous processes will be more resistant to the development of post-traumatic stress disorder. The persistence of nervous hypersensitivity in the limbic system, resulting from the experience of various environmental hazards, may be the reason for the individual's formation of anxiety as a trait. Anxiety and neurological hypersensitivity can therefore be created in parallel and be just another manifestation or other name for the same unit characteristic. Anxiety and impulsiveness are the basis for the formation of personality traits such as neuroticism and extraversion. Apart from impulsiveness, extraversion connects with low level of anxiety, whereas neuroticism with high level of anxiety. It has been assumed that extroverted persons will be more resistant to the formation of post-traumatic stress symptoms, and neurotic persons - particularly susceptible to the development of this disorder [7].

As a result of the already existing disorder which is stress after a stressful situation, emotional support is an extremely important and fundamental factor. One of the forms of this support - empathic listening - can have therapeutic value for victims when they experience intrusive images and feel compelled to talk about their experiences, which is the way to overwork them. However, when the victim calls up thoughts of an accident for too long, it can reinforce the compulsion to process. It is important then to encourage her to other activities. This introduction of new experiences can help the victim to combine new, fresh memories with traumatic memories. After a few days of the accident, cognitive assistance becomes more effective, in which the victim attributes the accident to the accident and overworked wrong beliefs about guilt or responsibility for what happened. Immediately after the accident the possibility of intervention is limited, however, within 24-72 hours after the event, formal therapeutic meetings, conducted by specialists in the field of mental health, can be held. Such meetings may positively influence the subsequent healing. They consist in establishing an orderly pattern in which the victim recalls the course of the event and expresses feelings and reactions related to it. She is also informed about symptoms that may occur. In group meetings there is an exchange of observations regarding the accident and more information is obtained about the typical nature of post-traumatic stress. They also become a source of mutual support. During a few days after the accident the victim usually can not stop thinking about him for a moment, he feels compelled to remember him again and again, he tries to remember the missing details, look for explanations and instructions. He also often wants to organize his knowledge, looking for cause-and-effect relationships and trying to understand the chain of events that led to the tragedy. People who work with victims of accidents should allow them to talk about the injury, to work on distorted beliefs about him, such as attributing guilt or deep guilt or shame embedded in them, helping them to get better. Such interventions may require frequent reinforcement so that the victim can internalize the new interpretation of the role played by the accident. It is important to regularly stop the memories of people who are "re-living" an event in order to avoid re-traumatizing memories. In this way, it is also provoked to impose memories of the therapist's inclusions on memories related to the accident [8].

Perspective of time perception as a method of PTSD treatment - an alternative to help victims of violence and crime

As has already been mentioned, post-traumatic stress disorder PTSD is an anxiety disorder caused by trauma. It is a drastic experience resulting from a terrible event that affects a person in a continuous manner, thus limiting the freedom and the ability to cope with everyday life. Negative memories that disrupt functioning and cause losing in the past, thus excluding the achievement of previously planned goals, objectives and accomplishments. There is no place for the present and the future, they have been completely supplanted by the tormenting past. The memory of man is based only on negative memories, an attempt to explain "what would happen if". Each subsequent day is not based on dreaming new ideas for life, change for the better, but only on growing distrust, fear, fear or vigilance.

The term itself - stress "post-traumatic" clearly indicates the past tense, something that has already happened, is behind us, has happened. Nevertheless, these people have stuck in the negative past without seeing the way of their existence. They can not move, break with the past, or learn to live with it. Thoughts and dreams of people suffering from PTSD are wrapped up in recurring nightmares that are difficult to free. Often these people, wanting to avoid their tormenting memories, get rid of everything that is related to the accident, catastrophe, and war. They avoid people, places and anything that can bring back bad memories.

The brain is the most complex organ in the universe. In his book, Incognito, the secret live of the brain, neuroscientist David Eagleman explains: "Your brain is built of hundreds of billions of cells called neurons and glial cells. Each of these cells is as complicated as a city [...] each cell sends electrical impulses to other cells [...]. The cells are connected to each other in a network of such a staggering level of complexity that human language turns out to be insufficient to describe it and a new variation of mathematics becomes necessary. [...]. If we include billions of neurons, it means that in one cubic centimeter of brain tissue there will be more connections than stars in the entire Milky Way galaxy. " It's really a huge number of connections. When we realize the extraordinary complexity and diversity of the brain, it is easier to understand why the multifaceted problem of PTSD is so difficult to grasp with the mind and root out of one's consciousness. When we are in dramatic circumstances, our state of consciousness changes: normal thought processes cease to function and management takes over an alternative operating system. This mechanism is extremely useful, if you unexpectedly find yourself in a situation where you have to run out of a building to save someone, or risk your life to get your co-workers out of the flames - your mind would think it's crazy. This altered state of consciousness is equally necessary when you fight to save your own life - regardless of whether you have fallen from a horse, been beaten by an aggressive spouse, or simply trying to survive another day in a serious dysfunctional family. However, the same system of brain chemistry that allows us to be heroes and survive extreme situations, imprinting traumatic events deep in our memory, emotions, consciousness and subconsciousness. Neurobiology proves that the brain does not store these moving memories in one place, but distributes them in many different locations: both in the area of the anterior cerebral cortex, responsible for thinking as well as in the area of the almond body and hippocampus responsible for emotions. The brain stores and codes for various types of memory traces - some of them are long-term, others are easy to remove using various types of chemicals. For example, the

details of yesterday's dinner can be saved with a pencil - easy to wipe and replace with the details of today's dinner. It may also happen that the dinner atmosphere will be inscribed on the list of important memories. The name of the person you just met or the place where you left your car keys yesterday can be saved with the disappearing ink in short-term memory. Traumatic memories, however, are like a tattoo - today you would prefer not to have it, but it is permanently fixed on your skin. Saved with eternal ink, they will always remain a part of long-term memory. This complex coding system means that traumatic memories, such as those accompanying PTSD, are multidimensional, insidious, difficult to track down and are difficult to deal with. Memories attack people suffering from PTSD in a way that deprives them of a sense of control, steals the future and makes them trapped in a terrible past and fatalistic present [9].

Balancing the perspectives of time perception described by the world-renowned psychologist Philips Zimbardo distinguishes six perspectives such as:

- past-negative,
- past-positive,
- present fatalistic,
- present hedonistic,
- she came,
- transcendental came.

The negative perspective of time perception is characterized by painful memories and experiences that have changed at a certain stage of life. These people return to the past, remembering their own mistakes, wrong choices in life, or taken such, and no other decisions. They wonder if they could do something different than the one they did. They complain about themselves, thereby introducing negative emotions among others.

The past positive perspective of perceiving time carries these good positive memories. In the memory of these people, the past was recorded as seeing good, warmth, family, friendship, and nice moments. Even in a situation that was not always comfortable, almost difficult to overcome, they can find a nice accent of those moments turning everything into a joke - they become optimistic and resilient. For such people, "the glass is always half full".

The present fatalistic perspective of perceiving time is characterized by a free attitude to life in which people entrust everything to their fate. Such persons are convinced that nothing depends on them, what is to be, it will be. They do not care about the consequences of their behavior, their decisions are unwise. They think that they have no impact on anything, and at the same time lose their sense of personal agency.

The current hedonistic perspective of perceiving time refers in turn to people who are creative, inventive, full of vigor and energy. Such persons are the opposite of those who live in the present fatalistic. They enjoy life and enjoy it beyond measure - fast driving, drug use, extreme sports. Often, balancing on the edge of risk. The motto of these people is "cerpe diem".

The future perspective of perceiving time concerns people who care about their future. They persistently strive to realize their own potentials, being cautious, aware of the consequences of their actions and behaviors. These people care about their health, avoid stimulants and constantly develop, thus wanting to achieve their goals or realize their dreams.

The transcendental future perspective of perceiving time is characterized by faith in eternal life. The soul is the most important, not the body. These people believe that only after death will they

experience peace, and human life is only a short episode on the way to salvation. They are fully devoted to God, prayer and contemplation.

The perspective of time is the foundation of the way people live. People tend to develop and abuse a specific perspective of time - for example, they focus on the future, the present or the past. People who are oriented towards the future usually have greater professional and academic successes, eat better, regularly exercise their body and effectively implement the program of preventive medical checks. "Late" students and other people who live in fast-paced communities are more likely to be future-oriented, with less willingness to devote their time to altruistic activities. On the other hand, people who are primarily oriented towards the present tend to be more ready to help others, but they seem less willing or able to take care of themselves. In general, the probability of taking risky sexual behaviors, gambling, addiction to alcohol or drugs is higher in people oriented towards the present than in people oriented for the future. Present-oriented people are also less willing to exercise, eat well, and take preventive health activities, such as cleaning teeth or regular medical check-ups. Consequently, future-oriented people have a better chance of succeeding, while the likelihood that they will help a person in need is smaller in their case. Paradoxically, people who have the greatest opportunity to help are at the same time the least willing to use it. The opposite is people focused on the present, who have less chance of success, but are more ready to help others. Another paradox is that individuals who are most ready to help others may be the least able to cope with themselves. The situation becomes more complicated when we consider people whose basic perspective of time perception is the past. For some, the past is filled with positive memories of family rituals, successes and pleasures. For others, the past is associated with negative memories, it is a repository of torments, regrets and losers. These divergent ways of perceiving the past play a crucial role in making everyday decisions, because they become binding reference systems, which are nurtured in their minds by those who preach positive or negative visions of the past [10].

People who have been victims of domestic violence, whether they have suffered a road accident or who have witnessed tragic events, often stop in their past - they are often unconscious. They try to analyze those events that recur to them cyclically, not giving them a fully normal function and live. These events cause post-traumatic stress disorder in them. Sometimes people blame themselves for finding themselves at a bad time and place, and yet they could do something else, be somewhere else. They blame themselves for their choice. The look seems to be irrational because man can not predict the turn of fate when he does not take risky actions. Each subsequent thought about the negative consequence of the intended action would lead man to behaviors taking the form of paranoids. Such behaviors are difficult to understand for a healthy person who was not exposed to dramatic situations, forcing sudden, unexpected actions. The life of such people focuses mainly on fear, fear and fear of the next day.

The perpetrator of domestic violence who abuses alcohol seems to need help only for him. He is the aggrieved because he fell into addiction. This brings him to antisocial behavior such as domestic violence. He does not realize, or does not want that the whole environment that surrounds him, the closest family can not fully function in fear of him. Family members are afraid of physical or psychological violence. People from families affected by domestic violence are losing themselves in this negative past. They think that nothing will change for good, they do not believe in their future. Often addicted to their torturer, they take life as it is. Focused on the problem that

everyday addicts bring to life, they forget about their needs, desires and dreams. They only commemorate these bad moments, these good ones are lost.

Similar behaviors affect people who have become victims of crimes against property or life. A person who suddenly meets an attacker threatening her with a dangerous tool in the form of a knife and demands a money release will behave in an instinctive manner. For fear of his life and health he will do the perpetrator's orders. Most of us in the place of this person will behave in the same way, but it will behave differently after the event. In each of us, this event will be stuck in the memory, but some people will remember it occasionally, tell their friends about it, and over time, though memories will remain, they will be remembered far and wide. They will not affect each day negatively. In others, this picture will be written deep in the memory and everything that is associated with that unlucky event will provoke strong fear and anxiety. Such associations can be the view of a knife, the place where the crime occurred, or a movie with a similar scene. The negative image that will be painted every day will overshadow the good moments and memories, and in the head people like a torn plate will return to uncertainty and uncertainty. One more important feature such as trust in people will be lost. The image that is built up against others is usually based on positive emotions, faith in humanity. This process takes years and, as a rule, people are perceived as good. In the situation of the offense of robbery, trust in others is severely shaken, and the faith in people is uncertain. These people can become fearful, unseen, or introverted. The negative situation makes these people forget that in the past on their way met many positive, helpful and polite people. One event changes so much.

Summary

Therapy in the perception of time not only becomes a help for war veterans who have experienced the cruelty of war, and the nightmare of those memories inscribed in their memory deeply enough that they are not able to function normally and in a healthy way. A proper understanding of the perspective of time perception and its balancing should also become a method of therapy for people from families affected by violence, both for the victims and the perpetrator. This method may also be appropriate for people who have suffered post-traumatic stress caused by a serious crime such as robbery or rape. People who are stuck in the negative past live in the fatalistic present - without ideas, goals or desires. They are not oriented to the future. Orientation for the future allows you to take care of yourself, have some control over what has been planned. It becomes an impulse for action, motivates for further challenges and I give faith in myself. Man should strive to improve the skills of living life that has been given to him. Focusing on the present moment, one should take into account the past and the future. Your present is being built on the foundations of your past. It is positive, if the past was positive - nice, positive memories about it. These people are healthier, happier, more successful. This, in turn, translates into a positive future, on the will to achieve development, plans and goals. This is a proper understanding of man. The same principle will have a negative past - the future in the image of such people will be meaningless.

You can not change your past. The events that took place were saved in memory less or more strongly. You can not erase it, but you can change your perception of the past, remembering and focusing on the positive things that have happened to us. Understand that fate may want to let us

experience these beautiful moments again. One event should not cross out our dreams that we had. The game of these positive situations in the past can not be buried and forgotten due to the time of one incident. The past can not be changed, but it can be formed - remembering and putting in the first place those situations and memories that provided us with joy and motivated us to continue our activities.

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CELL CULTURES AND ANIMAL MODELS IN RESEARCH ON WILSON'S DISEASE

Sylwia Szeląg-Pieniek[1*], Joanna Bujak[2], Patrycja Kopytko[2], Małgorzata Łączna[2]
[1] Zakład Farmakologii Doświadczalnej i Klinicznej, Pomorski Uniwersytet Medyczny w Szczecinie
[2] Katedra i Zakład Fizjologii, Pomorski Uniwersytet Medyczny w Szczecinie
* corresponding author: sylwia.szelag@pum.edu.pl

Abstract:

Wilson's disease (WD) is an autosomal recessive disorder associated with imbalance in copper homeostasis. It is caused by mutations in ATP7B gene which disables copper transport and excretion from cells. Copper accumulation causes numerous molecular, metabolic and morphological changes, resulting in liver damage, neurological impairment, as well as behavioural and psychiatric symptoms. Although WD is a well-known disease, there are still several questions to be answered for better understanding of its pathology. Cell cultures and animal models which mimic WD phenotype can provide very useful tools in research on the pathophysiology and development of new treatment methods of the disease.

Keywords:

ATP7B, Wilson's disease, mouse model, HepG2

Introduction

Cell cultures and animal models are among the most powerful tools used in medical research. They are considered as main sources of information about molecular biology, genetic, physiology and etiology of many different diseases in modern medicine. Initial steps in the research on development of new drugs, including toxicity tests, are usually conducted with use of cell cultures. The history of cell culture methods goes back to the end of nineteenth century but they gained major significance when HeLa cell line was developed in the middle of twentieth century [1]. Development of the immortal, fast-dividing cell line enabled an incredibly fast progress on fields of biological sciences and genetic engineering. Although investigations involving cell lines are relatively easy to perform and allow to obtain repeatable results, cells cultured outside the organism with no contact with internal environment, cannot exhibit the same features as cells inside the body. Therefore, different animal models are used in more advanced studies on physiology, pathophysiology and pharmacology. However, results of *in vivo* research conducted using animal models, albeit very valuable, cannot be easy translated to humans in some aspects. Therefore, knowledge on interspecies differences is substantial in order to create new models, allowing studies on pathogenesis and treatment of human diseases with different etiology [2,3].

Wilson's disease – etiology, manifestation and treatment

Wilson's disease (WD) is a rare genetic disorder inherited in an autosomal recessive manner. It was first described in 1912 by Samuel Wilson in his publication "Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver" [4]. The relation between Wilson's disease and copper accumulation had been suggested in 1948 by Cumings, but first genetic mutations responsible for the disease were identified in 1993 [4-6]. Mutations associated with Wilson's disease are localised in a gene encoding copper-transporting ATPase (*ATP7B*), which plays a main role in incorporation of copper into ceruloplasmin and excretion of this metal via bile. ATP7B protein plays a crucial role in Cu homeostasis in human and animal tissues, especially in liver where its expression is the highest from all the organs. The failure of copper transport and excretion from liver cells leads to accumulation of free Cu, and in consequence results in liver damage. During this process, free copper is also released to extracellular space and it is subsequently transported by plasma to other organs. Intoxication caused by excess of free copper is mainly manifested by liver impairment, cognitive and behavioural disorders and neurological symptoms [7,8].

Since liver is a main organ responsible for Cu metabolism in human body, it is very often the first one which is exposed to toxic effects of copper excess. However, first symptoms of Wilson's disease manifest as a liver failure only in approximately 40-60% of patients. Other patients initially manifest neurological and psychiatric symptoms. Patients with WD differ in onset time of first symptoms, as well as in symptoms intensity [8,9]. Wilson's disease patients were investigated for potential correlation of different *ATP7B* gene mutations with disease phenotype. However, the obtained results were inconsistent. That suggests that other, mutation-independent factors may play a role in modulating the WD phenotype. Moreover, some *ATP7B* mutations may show dual behaviour (pathogenic, resulting in disease onset, or non-pathogenic), depending on the population studied [10].

Cell cultures in research on Wilson's disease

Cell cultures are very often utilized in research on WD, in order to assess the function of copper metabolizing and transporting proteins [11-13] and predict the impact of *ATP7B* mutation, as well as different substances on ATP7B protein activity.

Human liver carcinoma (HepG2) cells are frequently used to investigate the effect of *ATP7B* mutations on copper transport and treatment effectiveness. In 2016, Chandhok et al. [14] used HepG2 cells expressing different *ATP7B* mutations to study the effect of anti-copper treatment on intracellular trafficking, viability, and apoptosis. The authors showed that hepatic cell lines carrying WD-related mutations can be a proper tool for analysis of the basic impact of specific genotype on cellular changes in Wilson's disease [14]. Effects of various *ATP7B* mutations on intracellular localization of the encoded protein were also investigated in another hepatocyte-derived carcinoma cell line - HuH-7 [15]. Other studies were focused on the most frequent pathogenic mutation within *ATP7B* gene: H1069Q, which causes the retention of WD-protein in endoplasmic reticulum (ER). Thus, although potentially able to transport Cu, that particular ATP7B variant cannot reach Cu excretion sites to remove excess copper from hepatocytes. There is currently no cure for WD, and the main option is symptomatic therapy with chelating agents, which bind copper and increase its

renal elimination. Better understanding of mutation effects and mechanisms which may impact WD-protein functions may lead to future development of novel, more efficient therapeutic strategies [16]. Beside hepatoma cells, HEK293 cell line has been frequently used to investigate the *ATP7B* mutations effects [17,18]. That cell line, derived from human embryonic kidney is routinely used as a model in expression studies on many recombinant proteins [19].

Human cell lines are often used in research on molecular consequences of mutations and polymorphisms in disease-related genes. In case of WD genetic variants, it was revealed that the change in the amino acid sequence of *ATP7B* may show a dual behaviour in various conditions. Gupta et al. used HEK293 cells and Menkes fibroblasts transfected with tyrosinase in their investigation on intracellular targeting and activity of ATP7B-Arg^{875} variant of *ATP7B* [20]. The authors showed that the Gly^{875}→Arg substitution within the A-domain of *ATP7B* decreases stability of that region. Unexpectedly they also demonstrated that the elevated copper level can stabilize folded mutated ATP7B protein and restore its transporting properties. That study showed that not only mutation type but also nongenetic factors may play crucial role in WD pathogenesis and disease phenotype [20].

Because of great variation of disease course and progress in WD patients, some other factors beyond the *ATP7B* mutations are considered as responsible for the observed WD phenotype. Among them, there are proteins interacting with ATP7B and taking part in Cu transport [21]. Those proteins can affect intracellular copper level, so they can additionally influence Wilson's disease phenotype, irrespective of the mutation type in WD-protein.

Beside the examples of genotype-phenotype studies presented above, HepG2 cell line is also commonly used in evaluation of an impact of different substances on ATP7B activity. For example, Berzina et al. studied the influence of curcumin on copper transport and excretion in HepG2 cells carrying wildtype ATP7B [22]. They demonstrated that curcumin may stimulate intracellular copper transport in HepG2 cells. On the other hand, curcumin leads to copper accumulation in studied *in vitro* model. Moreover, curcumin accelerated copper absorption into HepG2 cells unless the cells have already absorbed toxic levels of copper.

HepG2 cells are widely used in numerous studies related with copper transport. Nevertheless, it should be taken into consideration that HepG2 is a cancerous, immortal cell line. Even if cells' morphology is similar to normal human cells in several aspects, there are many differences, i.e. in gene expression and cellular pathways related to cell cycle and apoptosis. As a consequence, observations conducted may not mimic properly human physiology, and the results obtained by means of that model are much less predictive, when compared primary human hepatocytes. [23,24].

Most of human cell lines, that were used in basic studies focused on various *ATP7B* mutation effects and intracellular interactions of mutated protein are commercially available. Obviously, those cell lines require additional modifications like site-directed mutagenesis, DNA transfection, RNA interference or other procedures allowing to adjust cell culture to researcher requirements. Nevertheless, to better understand the mechanism of liver damage in individual patients it would be the best to use patient's own liver tissue in analysis. However, primary hepatocytes obtained from patient's liver cannot be considered as a universal model system for WD studies. Primary cells are not only difficult to obtain but also culturing of them is very problematic due to the lack of proliferation ability. In addition, primary cells rapidly undergo apoptosis and can be already

damaged at the moment of liver biopsy [25]. However, the discovery of induced pluripotent stem cells (iPSCs) can possibly overpass those problems and enable *in vitro* studies on cells genetically identical to patient's 'original' tissues [26].

Zhang S. et al. reprogrammed fibroblasts obtained from patients suffering from WD to iPSC and subsequently differentiated them into hepatocyte-like cells [27]. The obtained cell model was characterised by copper-export defect, which is the main phenotype feature of WD liver cells. The authors suggested that hepatocyte-like cells from WD iPSCs may be a valuable tool for studying signalling pathways activated by accumulated copper and an inflammatory response [27]. Hepatocyte-like cells derived from iPSCs have been also used by Parisi et al. [28]. They used the generation of human iPSCs from patients bearing the H1069Q ATP7B mutation to investigate cellular localization of mutant protein. The obtained results suggested that the major cause for loss of ATP7B function in H1069Q patients was rapid ATP7B protein degradation. That research may trigger further studies, aimed at development of novel therapeutic strategies to rescue ATP7B-H1069Q function and initiate work on new drugs for WD patients [28].

Animal models of Wilson disease

Animal models of WD are significantly more advanced than cell line models. Observations based on the living organism, harbouring a functional mutation provide more informative data, because they take into account the complexity of pathogenic processes occurring in the body. There are several animal models that are used in studies on mechanisms of WD pathogenesis, including three main rodent models used in WD research.

Mouse models

First of them is the "toxic milk" (tx) mouse. Those mice suffer from inherited copper metabolism disorder caused by point mutation in *Atp7b*, changing methionine to valine at position 1358 [29]. The result of this substitution is a loss of copper transport function that leads to Wilson's disease-like phenotype in tx mice [30]. Beside liver damage, this animal model of WD manifests neurological and cognitive changes during disease progression [31]. Moreover, disturbed intracellular localization of the ATP7B protein is responsible for a low concentration of copper in the milk of the tx mice. It results in copper deficiency in mutants' pups and their early death as long as they are fed by tx mothers [32]. Toxic milk mice provide a good model for testing of new therapeutic agents for treatment of Wilson's disease [33]. Moreover, they can be used as an alternative animal model of metabolic liver disease requiring cell transplantation [34].

The second commonly used mouse model of WD is Atp7b knockout mouse (KO mouse/ $Atp7b^{-/-}$). This model is characterized by complete knockout of Atp7b protein, resulting in elevated copper levels in liver. Copper accumulates in the livers of those mice to reach the highest concentration at 6 weeks of age, and phenotypical and metabolic changes are observed from 12th week. The liver phenotype is more severe in this model compared to tx mice, however, KO mice do not manifest neurological symptoms of copper poisoning. Similar to tx mice, also KO mice show significantly decreased copper levels in mutants milk in comparison to control group [35,36]. That model was used to characterize changes in the hepatic mRNAs, proteins, and metabolites in response to copper accumulation [37]. The authors observed that elevated copper affects specific cellular targets in $Atp7b^{-/-}$ mouse livers, as well as in WD-patients livers, leading to lipid metabolism disruption. They

also suggested that these molecular and biochemical changes, but not oxidative stress, were responsible for major effect of copper excess at the early stages of the disease [37]. Additionally, Hamilton at al. [38] demonstrated that down-regulation of cholesterol biosynthesis is caused by inhibition of signaling mediated by nuclear receptors, especially the LXR/RXR heterodimer. They also suggested that LXR agonists could be potentially used in treatment to improve liver function, what would provide another option in WD therapy. Atp7b knockout mouse have been also used in gene therapy experiments. It was demonstrated, that phenotypical improvement of lipid biosynthesis and liver copper concentration may be achieved by liver-targeted transduction with *Atp7b* cDNA, followed by normalization of liver histology in *Atp7b* KO mice [39,40]. Nevertheless, despite these promising results, application of gene therapy in WD needs to be further extensively studied before this method could be potentially used in humans.

Rat model

Long-Evans Cinnamon (LEC) rat is the third of the most frequently used animal models of Wilson's disease. This model is widely used in WD pathophysiology studies. LEC rats are characterized by deletion at the 3' end of *Atp7b* gene, resulting in protein function impairment [41]. LEC rats share many clinical attributes of human WD, for example impaired copper incorporation into ceruloplasmin, disrupted excretion of Cu into bile and dramatically increased hepatic copper concentration, leading to hepatitis and spontaneous acute liver failure [42]. Kadowaki et al. examined changes in gene expression during progression of acute hepatic injury. They concluded that activation of p38 mapk in the livers of LEC rats may be related to progression of acute hepatic injury in these animals. They also suggested that inhibition of p38 mapk by specific inhibitors may attenuate acute hepatic injury in patients with Wilson's disease [43].

Because the acute liver failure is life-threatening condition, it is clinically important to prevent its occurrence in patients. For the last couple of years, microRNAs are taken into consideration to be utilized as a specific markers of liver failure. It was noted that elevated levels of liver-specific miR-122 in serum may be a good biomarker of liver diseases and liver damage. Using the LEC rat model of WD, Siaj et al. demonstrated that serum miR-122 can be a non-invasive, high specific tool to diagnose severe liver disease, as well as to monitor the course of inherited liver diseases [44].

Beside the liver failure, LEC rats show also other phenotype features of WD. Fujiwara et al. assessed memory, cognitive and motor functions of LEC rats in comparison to Wistar rats. Their results showed that LEC rat exhibited behavioural abnormalities similar to symptoms occurring in WD patients [45]. That suggests, that Long-Evans Cinnamon rat can be used as a liver pathology model of WD as well as neurologic model of the disease.

Similarly to mice models, LEC rats have also been used in investigations on novel therapies. In 2014, Chen et al. investigated whether transplantation of ATP7B-transduced bone marrow mesenchymal stem cells could decrease copper overload in LEC rats [46]. Despite promising results, authors pointed to potential problems, limiting potential application of the tested method in humans.

Other animal models

Recently, Jiang et al. have used the CRISPR/Cas9 system to create a rabbit model of Wilson's disease with a point mutation introduced into the rabbit *ATP7B* gene [47]. Additionally, Labrador retriever considered as a future animal model of WD, as similar genetic mutations are present in

both humans and dogs, leading to copper toxicosis [48]. Due to larger body size and a long life span (compared to rodents), Labrador retrievers may provide an alternative option in research on Wilson's disease. Searching for new animal models of WD with a desirable, precisely designed phenotype, could open possibilities for testing of therapeutic strategies and gene therapy research.

Conclusions and perspectives

Wilson's disease is a rare genetic condition, characterized by heterogeneous course in individual patients. There is still limited understanding of many aspects of Wilson's disease, for instance differences in an onset time and variety of symptoms' severity between patients. As a consequence, currently available therapeutic options are far from satisfactory, only symptomatic, and their efficacy is very limited. Hence, development of new, more informative models of WD seems crucial in order to achieve effective disease treatment in the future.

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