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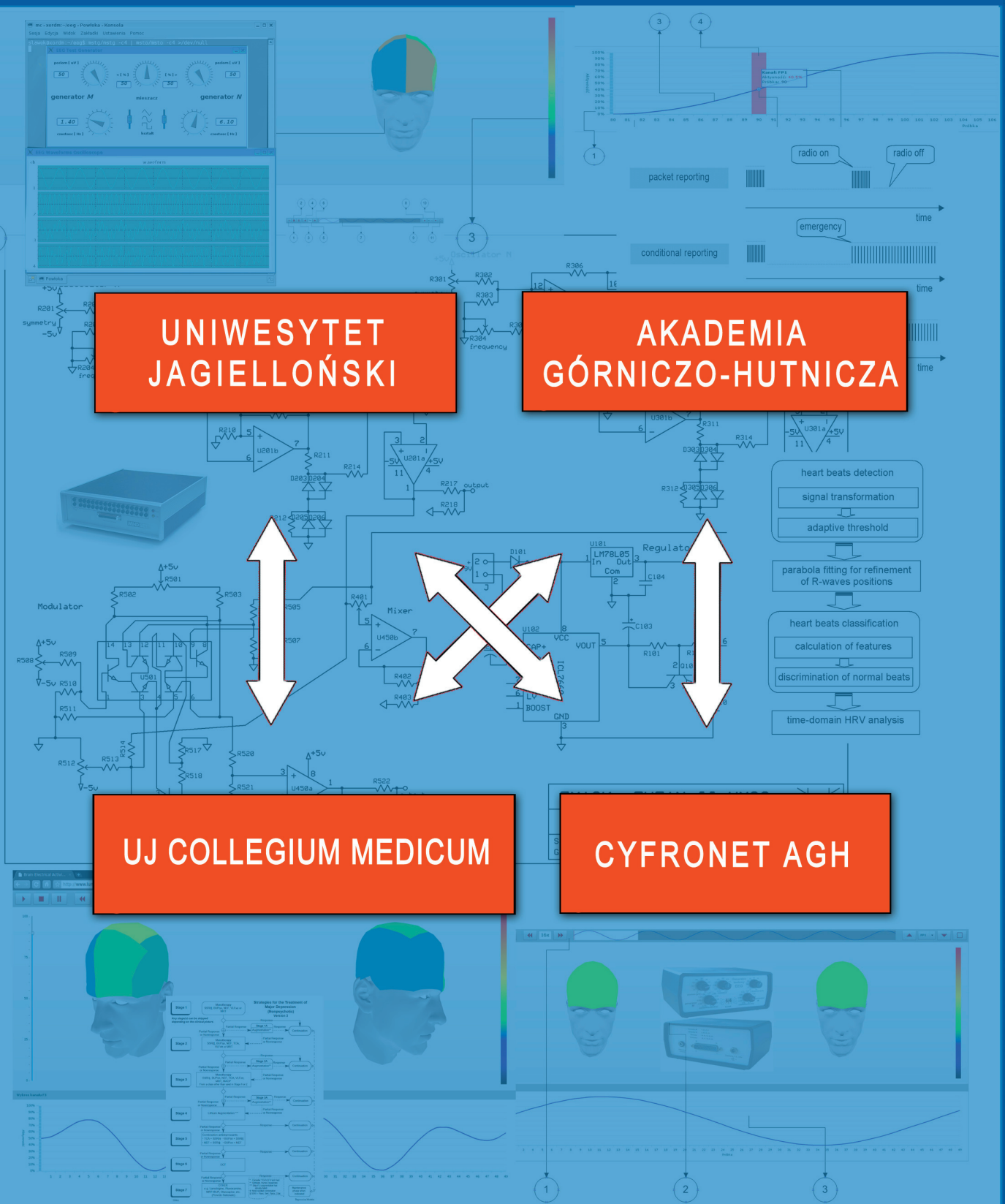

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PERSONAL WEARABLE MONITOR OF THE HEART RATE VARIABILITY

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Abstract: The aim of the paper is to present a prototype of wearable heart rate variability (HRV) monitor. The home care surveillance and sleep assessment system is partly embedded into a smart home infrastructure and partly worn by a supervised person. The prototype wearable device is designed to acquire and process the electrocardiogram and to send reports accordingly to a programmed schedule. The recording, processing and transmission modes are programmable, what allows the recorder to respond immediately in case of predefined thresholds excess, while the regular reporting is organized in delayed packets exchanged during a short transmission session. This approach significantly reduces the contribution to the total power consumption from the communication module. The prototype was based on the PXA-270 development kit, but due to very low power consumption (0,5 mW) the migration to a more compact system is considered.
Keywords: sleep monitoring, assisted living, wireless body area network, heart rate variability

Introduction

Wireless monitoring of basic vital parameters is currently one of the hottest research areas and prospective application fields as assisted living and home care. Current experiences show, that personalization of devices helps in refinement of distributed diagnosis calculation. First home care implementations prove that numerous diagnostic devices restricted to clinical use so far are applicable as elements of home automation increasing the users safety. Their role in the prevention or follow-up is doubtless and among other aspects brings considerable reduction of medical costs. Thanks to deep modulation of functionality achieved with the use of software and remote configuration they fit into the paradigm of personalized medicine.

The aim of the project was to built a prototype of home care surveillance and sleep assessment system including a wearable heart rate variability (HRV) monitor. The whole system is a hybrid partly embedded into a smart home infrastructure and partly worn by the supervised person. Both components are personalized by the software settings and dynamically linked executable code. The prototype wearable device is designed to acquire and process the electrocardiogram and to send reports accordingly to a programmed schedule.

The ECG-derived HRV is commonly recognized as easily available representation of autonomous nervous system (ANS) and clinically studied in pursuits for many neurological disorders [1-2]. Currents studies show that the HRV analysis in sleep is particularly meaningful. Because of lack of the voluntary actions of the subject in sleep, all heart rate accelerations and decelerations may be interpreted as ANS-related. The advantage of heart rate analysis is a wide range of data acquisition methods. The tachogram is usually derived from the single lead ECG signal, but upon the necessity the pulseoximeter, the arterial pressure meter or the cardiac microphone may also be a reliable source of RR interval series. The ECG-derived tachogram is a low data rate signal (12 bits per second) what suggests the ECG processing to be performed directly by the embedded software of a wearable recorder. Thanks to the low data-streams, the transfer may be organized in sessions allowing for considerable power savings or through a seamless link when immediate system response is needed. In the interpretation of the HRV common time- and frequency domain parameters were used. This facilitates data referencing to the clinical results in both: development and operating stages. The algorithms for tachogram processing are more complicated [3-4], particularly due to the sampling normalization required for spectral analysis. In the sleep assessment system the HRV parameters are interpreted with consideration of simultaneously recorded subject motion and acoustic signal described by snoring-specific parameters.

The design of an ECG recorder worn at sleep must particularly consider the factor of usage comfort, therefore the weight, size and autonomous operation time are crucial parameters.

Therefore several energy savings-oriented measures were taken into consideration at the design stage. Besides the use of low-power circuitry, main contribution to the longevity of the wearable recorder are: optimization of the ECG processing and organization of the wireless transfer of tachogram series. Unfortunately, due to the required mobility of the subject implying the omnidirectional radio wave propagation, the wireless connection is the main contribution to the power consumption.

Materials and methods

Deriving the tachogram from the ECGrecord

The heart rate reported or displayed for diagnostic purposes is usually the average value of seven consecutive RR intervals. Excluding two shorter and one longer intervals from the averaging increases the HR report stability in the presence of missing or extra detections. The instantaneous HR and its beat-to-beat variations (HR variability, or HRV) have been studied for many years, particularly in the framework of investigation of the ANS balance. In periods of stable HR, there are small beat-to-beat variations that result from the balance between the sympathetic system and the parasympathetic system. For this reason any pharmacologic agent that acts over ANS functions also influences the heart rate. Lower rhythms (sinus bradycardia, below 50 bpm), resulting from the prevalence of a parasympathetic system, occur in states of deep relaxation or in athletes with enlarged stroke volume. Higher rhythms (sinus tachycardia, above 110 bpm) are caused by the dominance of a sympathetic system and appear during physical effort or mental stress. The time-domain parameters of the heart rate variability are thus crucial indicators for the human surveillance in everyday life including possible assessment of sleep disorders. Since the ANS influences mainly the sinoatrial node (SA) in the heart conductive system, the HRV parameters aiming at assessment of the ANS require to be calculated from the NN (normal-to-normal) time intervals. In case of occurrence of escape or complementary stimulations, these beats need to be excluded and replaced by a hypothetical heart beat of sinoatrial origin.

The ECG record needs specific processing to yield the RR time series. This includes the temporal localization of the heart beats and basic classification of beat types. Single-lead electrocardiogram is sufficient to provide a reliable temporal markers and usually also information of origin for each heart beat. The use of three bipolar lead system (Holter type) is considered, but not implemented in the prototype for the reason of subject's comfort. The ECG is acquired with the sampling frequency of 500 sps and 12 bits resolution. The processing is performed in real time with the use of mathematical signal transformation revealing the common features of the QRS complex. Next, the adaptive threshold is applied to discriminate the QRS section. The localization of the R wave peak is further refined to 1 ms with use of five points-based parabola fitting [5]. Beat origin labeling needs further processing of the signal sections isolated in 100 ms vicinity of the R wave peak. Certain features of the signal most discriminative for atrial and ventricular beats [6] are calculated and support the final decision on the beat origin attribute. The beats are next qualified to the time-domain HRV analyzer. The whole processing yields for each heartbeat a pair of numerical descriptors aggregated in a word:

- the relative position coded in 12 bits,
- the origin coded in remaining 4 bits.
- For the reason of data integrity one beat type is reserved for a synchronization time marker.

Since the ECG interpretation algorithm is embedded and raw ECG record is not available in normal operation mode, the performance of the processing and pertinence of the sleep analysis rely on the robustness of the beat detection and classification methods to interferences and noise. The algorithm applied as recorder's embedded software was developed as a part of real-time Holter interpreter and complies with the essential performance requirements (IEC60601-2-51). The wearable device is thus considered reliable in standard recording conditions, therefore particular attention should be paid by the user to the signal quality and electrodes placement. Although the implementation of advanced methods for signal recognition is technically possible, extending the range of usage conditions causes the increase of computational complexity and shortens the autonomy time.

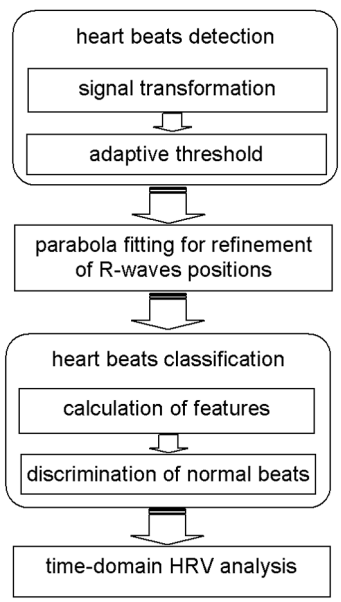

Fig. 1. Scheme of embedded ECG interpretation algorithm

Dataflow organization

The recording, processing and transmission modes are programmable, what allows the recorder to respond immediately in case of predefined thresholds excess. The embedded time-domain HRV analyzer provides values for decision on the dataflow mode (fig. 2). The available modes are:

- packet reporting for off-line transfer of the HRV data,
- conditional reporting which switches between the packet and the seamless data transfer accordingly to specified conditions on the HRV parameters value,
- seamless reporting for the time-synchronized transfer of the HRV data.

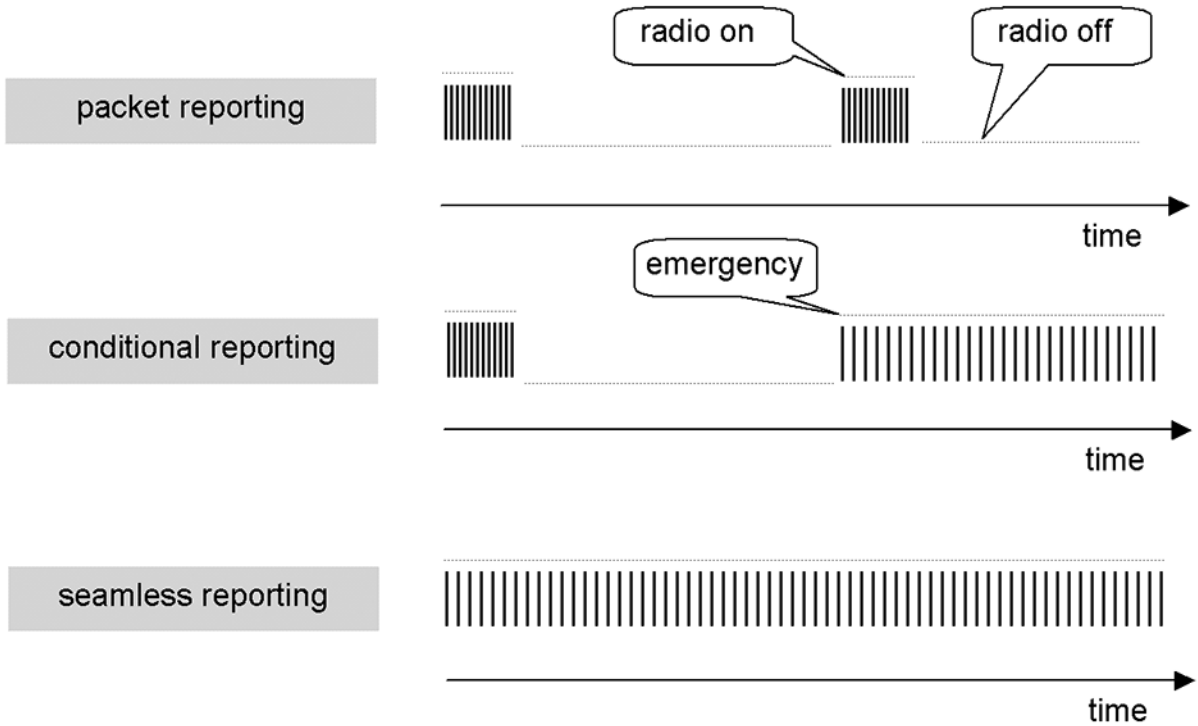

Fig. 2. Summary of the HRV data reporting modes

In first (regular) reporting mode the data are stored in a buffer of predefined length (300 – 3600s) and organized in packets exchanged during a short transmission session. This mode provides delayed information about the subject's state, but significantly reduces the contribution to the total power consumption from the communication module.

In the conditional reporting mode, depending on predefined settings and actual parameter values, the data are stored in a buffer and reported in packets in a given time interval or transmitted immediately to the Bluetooth interface of house-embedded monitoring system. Programmable parameters are:

– epoch length – determining the epochs averaging time and thus the borderline between the fast (RMSSD) and slow (SDANN) ANS components (usually set to 5 min),
– tachycardia and bradycardia limits – determining the possible continuous reporting mode (default values are 110 and 50 bpm respectively),
– local variation limit – determining the possible continuous reporting mode in case of HR instability,
– abnormal beats percentage limit – determining the acceptable contribution from non-sinus rhythms.

In case of continuous monitoring, the report content is also specified by the software settings and may range from the raw ECG to the tachogram and HRV parameters. In this mode the surveillance and sleep assessment system reads the subject's HRV data simultaneously with the results of other measurements.

Prototype design

The target device is designed as a lightweight and reduced in size (below 1 sq inch) and provides a Bluetooth wireless interface and a mini-USB connector. It is powered by a built-in Li-Ion accumulator charged via USB. The prototype was based on the PXA-270 development kit (fig. 3), but due to very low power consumption (0,5 mW) the migration to a more compact system is considered. Initially we used the commercially available ECG recorder (Aspekt500, Aspel) as the subject's front-end, but this 12-leads recorder was replaced by a single-channel custom-built module with similar measurement specification (full range $\pm$12mV, noise 1μV RMS, k_u = 85V/V), but with significantly reduced power requirements (0,5mV). Further reduction of the power consumption was studied, but below the given value, the amplifier was increasingly prone to interferences.

The host surveillance system was based on the task-oriented analysis of the video and audio data and mounted in a dormitory ceiling. The embedded system provided a Bluetooth interface for a two-way digital communication with the wearable monitor of subject's HRV parameters. The surveillance system was designed for studies on sleep quality and detection of the apnea and other sleep disturbances. Recent interpretations show the correlation of the subject's motion with HRV parameters as an important health factor.

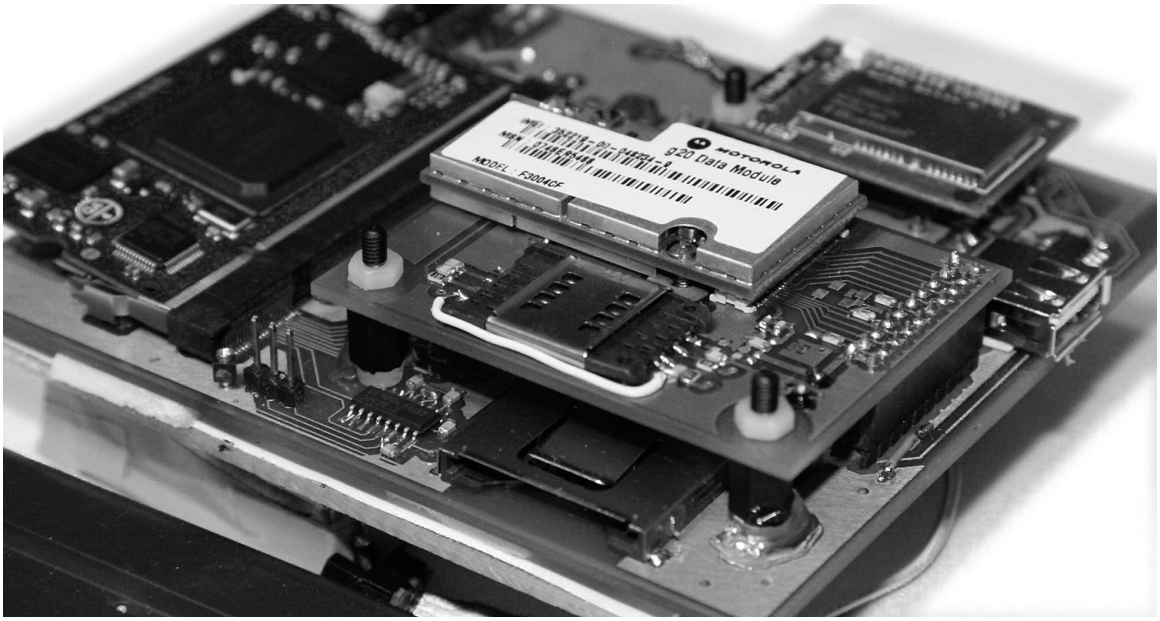

Fig. 3. Prototype wearable recorder with wireless interface

Testing procedure

The HRV recorder was tested for the conformance with a conventional HRV analysis with use of selected (best) lead of the MIT-BIH Arrhythmia Database [7], resampled to 500Hz. The reference positions of the heart beats and the beat types were verified by a commercial Holter analysis software (Holcard 24W, Aspel).

– The validation of the embedded software was performed first and used digital ECG records. The output RR sequences acquired from wired USB connection was compared to the reference provided by the commercial software.
– The overall verification of the wearable device includes the participation from the analog circuitry and wireless communication module. For these tests the corresponding ECG signal was generated in the analog form (amplitude scale 3.9μV/LSB, fp 200Hz, output impedance 500Ω). This signal was captured, interpreted and the result RR sequences provided by a wearable reorder via Bluetooth interface were compared to their references.

Results

Results of the comparison between two RR interval series and heart beat annotation are presented for software and overall tests in table 1 and 2 respectively.

Complementary results reveal the benefits from the applied data transfer modes. The Bluetooth module requires 160 mW of power for operation, while only 3.1 mW in standby mode. For the HRV data packet collected in one hour (9 kB) the measured duration of the transmission session is 7 s (including the connection setup, confirmation and backward settings of parameters). The average power consumption in the longest packet reporting mode may thus be significantly reduced from its initial value of 161 mW to 1,3 mW i.e. by 99.2%. The corresponding extension of subject's device autonomous operation allows for 230h instead of 1,8h in seamless reporting.

Tab. 1. Difference of RR intervals between the tested and commercial interpretation software, and the sensitivity and specificity of normal heart beats labeling (testing of the embedded software)

MIT-BIH ID	**avgDRR** [ms]	**stdDRR** [ms]	**sens NN** [%]	**spec NN** [%]
100	5.7	3.1	98.8	99.1
101	6.6	4.7	99.1	99.2
		.....		
234	12.1	7.1	97.3	95.4
Average	**6.83**	**5.37**	**98.5**	**97.9**

Tab. 2. Difference of RR intervals between the tested and commercial interpretation software, and the sensitivity and specificity of normal heart beats labeling (overall verification of the system)

MIT-BIH ID	**avgDRR** [ms]	**stdDRR** [ms]	**sens NN** [%]	**spec NN** [%]
100	10.1	5.3	98.7	99.0
101	9.3	4.9	98.9	99.0
		.....		
234	13.1	9.1	96.9	95.0
Average	8.17	6.96	98.3	97.7

Conclusion

A simple diagnostic system for the assessment of the human ANS in motion and during the sleep was designed and tested. The system is based on HRV parameters calculated on an ECG--derived tachogram. Thanks to the use of a single recording channel and simplicity of the embedded signal processing, the target system can be shrunk to less than 1 sq inch, what allows for integrated mounting on the electrode. The recording of heart rate is continuous, however significant extension of autonomy time (up to two hundred hours) is achieved thanks to the programmable organization of the transfer. Differences between the custom-built and reference algorithms expressed in tables 1 and 2 originate from different detection technique, low sampling frequency and unknown RR intervals averaging applied in the commercial software.

In author's opinion, the principal benefit of the system comes from the possibility of conditional reporting based on on-board calculated HRV parameters.

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E-LEARNING IN PHARMACEUTICAL CONTINUING EDUCATION IN POLAND

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Abstract: E-learning systems have become important tools in the process of continuing education of pharmacists and other healthcare specialists. In Poland the continuing education of graduated pharmacists is obligatory in order to keep their professional license valid. Therefore pharmacists receive credits by taking part in various courses, training and conferences. Nowadays more than 50% of Polish professionally active pharmacists, which means above 11,000 professionals, take part in courses organized with use of e-learning platforms. There are three available and independent from each other systems. e-duk@cja is the largest and most developed system dedicated to pharmacist continuing education. The first version of the Internet based learning system e-duk@cja was launched in February 2005 and was a common project of the Krakow Local Pharmaceutical Chamber and Faculty of Pharmacy Jagiellonian University Medical College. All twenty Local Chambers accept courses and certificates granted by validated on the national level Centre of Postgraduate Education managed by the Faculty of Pharmacy in Krakow. Before being published, learning materials need to be approved by the Scientific Committee. All these courses including testing procedure are provided online free of charge. The aim of the e-duk@cja project is to develop a distance learning system for the life-long educational needs of pharmacists. For today e-duk@cja has more than 11,000 active users, 18 courses, 32 credits, ca. 600 independent users' visits daily and above 2 million hits per year. The newest development was chosen on the functionality and practicality level and includes automatic certification and ready-for-print certificate generation. Other platforms include farmacja.edu.pl, which was created by educators from the Faculty of Pharmacy Warsaw Medical University. The recently established portal e-umed.lodz.pl is provided by Medical University in Łódź. The courses are directed not only to pharmacists but also to physicians who are involved in the continuing education process as healthcare professionals. Constant increase in users' number proves that this kind of pharmaceutical vocational development is an important element of postgraduate education.
Keywords: Pharmacy continuing education, e-learning, online learning, Internet based learning, professional education

Introduction

Due to the ubiquity of the Internet, e-learning is becoming more and more important tool in education. "e-learning is an approach to facilitate and enhance learning through, and based on, both computer and communications technology. […] may be used to suit distance learning through the use of Wide Area Networks, and may also be considered to be a form of flexible learning where just-in-time learning is possible" [1]. This wide definition comes from wikipedia.com, the site prepared by people who work and learn together [2]. Others describe e-learning as simply pedagogy empowered by digital technology [3].

Nowadays e-learning is defined as the acquisition of knowledge and skills using electronic technologies such as computer- and Internet- based courseware and local and wide area networks [4]. It cannot be forgotten that e-learning should involve student-student, student-teacher or teacher-teacher interaction. Participants of e-learning courses should be aware of the results of their education process; their knowledge should be evaluated throughout the course. Therefore uploading materials on a website, like lectures or exercises, is still not real e-learning since it lacks this interactive component [12].Today e-education is often linked with traditional learning. This leads to the formation blended learning, Europe's first e-learning initiative [2]. This method combines advantages of traditional learning with advantages of e-learning and some of them involve:

– wide spectrum of subjects available,
– cost reduction,
– time flexibility,
– integrated assessment tools,
– multimedia materials,
– high interactivity [2].

Advantages of traditional learning involve:
– direct interpersonal relations,
– live contact with the tutor,
– well defined time and place of training,
– evaluation of knowledge,
– contact with real experiment,
– training of interpersonal ability [2].

Pharmacists continuing education with use of e-learning approach

Distance learning is wide spread and well developed in large countries like USA, Canada or Australia.There are already a lot of e-learning platforms for pharmacists providing continuing education in the Internet. In the United States Post-graduate Healthcare Education (PHE) provides Power-Pak C.E.® educational programs for the broad spectrum of healthcare professions. Professional medical and pharmaceutical education constitutes PHE's core activity. This mission is achieved through the company's ongoing continuing education activities. The primary goal of all continuing education programs is to provide, promote, and disseminate topical education to all medical personnel and pharmacists in order to maintain and improve their knowledge and skill. To participate in Power-Pak C.E.® online courses, a user needs to read the course he is interested in and submit his answers to the questions. Participants receive instantaneous grading of their exam. Upon successful completion of an exam (a score of 70% or higher), a certificate is generated automatically. Power-Pak C.E.® online customizes its website for each visitor by creating a personal participant profile. Registered participants may update their contact information, take an exam, receive instant grading, view their exam history, and print certificates for successfully completed programs at any time. Monthly notifications are sent to users notifying them of new courses available on the site [9]. Courses are granted with Continuing Education credits accredited by the Accreditation Council for Pharmacy Education. All the online courses are stored on the web site: www.powerpak.com.

RxSchool is a unique e-learning solutions company that successfully connects healthcare professionals with leading providers of educational content. Powered by the RxSchool Learning Management System, this web site hosts one of the largest libraries of the Accreditation Council for Pharmacy Education accredited continuing education on the Internet. Many of the courses available here are free of charge thanks to the collaborative efforts of "RxSchool Powered" providers, educational institutions, employers, associations and pharmaceutical industry sponsors. The courses can be found on the site: www.rxschool.com. RxSchool offers also Live Internet education available through the popular RxSchool Live seminar room. Its leading Internet delivery solution provides healthcare professionals with a convenient way to get quality live education that qualifies for live CE credit [10].

Another popular e-learning portal for healthcare professionnnals is Free CE. Since 1996 Free CE has been giving access to free, quality based, continuing education. A lot of live webinars/web casts and self study monographs are provided by this platform. Free CE is a subsidiary of PharmCon, Inc, the Accreditation Council for Pharmacy Education approved provider of continuing education. PharmCon specializes in the accreditation and coordination of live continuing education programs [11].

Pharmacists continuing education in Poland

According to Polish pharmaceutical law requirements, every practicing pharmacist must gain 100 educational credits during a 5-year period. The educational credit pool is sub-divided into two groups – those with and those without an exam [5]; so called *hard* and *soft* credits. These continuing education credits are necessary to extend the professional license for the next period []. Pharmacists need to collect at least 50 *hard* credits and 50 *soft* credits during a 5-years long period. The educational process remains under the control of accredited Centres of Postgraduated Education, working in cooperation with the Local Pharmaceutical Boards. Every pharmacist has his own "educational card" where the points are recorded [5]. The need to participate in the accredited educational courses to keep the professional license makes life-long learning obligatory for Polish pharmacists.

Unfortunately there are still some gaps in the legal regulations in the current education system regarding utilization of the e-learning techniques. This is the basic obstacle for the development of distance learning in Poland. Nevertheless the situation has improved recently. Constructing a didactic process with the use of Internet potentially requires the competent application of both educational regulations and baseline programmes at schools [2]. Another barrier is computer literacy. The majority of users confess that their computer activity is limited to simple applications, frequently created especially for beginners [2], but the situation is improving substantially.

e-Learning platforms for pharmacists continuing education

Nowadays there are three main e-learning platforms for pharmacists in Poland: farmacja.edu.pl, created by educators from the Faculty of Pharmacy Warsaw Medical University, a recently established portal e-umed.edu.pl provided by Medical University in Łódź and the oldest and, in our opinion, the largest platform e-duk@cja, established by the educators from the Faculty of Pharmacy Medical College Jagiellonian University in Krakow. The first version of the Internet based learning system for pharmacists e-duk@cja was launched in February 2005. At the beginning, participants of the e-courses were pharmacists only from the Local Pharmaceutical Board in Krakow. In following years pharmacists from other Local Pharmaceutical Boards subscribed to the system and nowadays there are users from all twenty Local Pharmaceutical Boards in Poland.

One of the assumptions at the planning stage was high scientific level of the offered educational material.To provide independent reviewers opinion, all courses before publication need to be approved by the Scientific Committee, which consists of three professors of pharmacy from the Faculty of Pharmacy of Jagiellonian University Medical College.

Users

On May 2010 the total amount of registered users of the system e-duk@cja was 11 500, which includes more than 50% of all registered pharmacists in Poland [6]. In average there are 300--500 users who log in daily depending on the week day. The

Visits	Pages/Visit	Avg. Time on Site	% New Visits	Bounce Rate
179,177	10.08	00:09:22	21.17%	19.16%
% of Site Total: 100.00%	Site Avg: 10.08 (0.00%)	Site Avg: 00:09:22 (0.00%)	Site Avg: 21.11% (0.28%)	Site Avg: 19.16% (0.00%)

	Detail Level: Country/Territory	Visits ↓	Pages/Visit	Avg. Time on Site	% New Visits	Bounce Rate
1.	Poland	176,548	10.09	00:09:22	21.14%	19.12%
2.	United Kingdom	823	10.43	00:11:33	16.40%	21.51%
3.	Germany	474	11.34	00:09:38	26.37%	8.23%
4.	United States	331	9.07	00:11:21	24.17%	28.10%
5.	(not set)	187	8.12	00:08:47	20.32%	17.11%
6.	Switzerland	139	9.42	00:09:18	15.11%	8.63%
7.	Ireland	128	6.73	00:06:07	28.91%	34.38%
8.	Sweden	85	7.86	00:05:42	24.71%	29.41%
9.	France	72	11.19	00:07:46	16.67%	16.67%
10.	Norway	61	11.56	00:09:34	29.51%	22.95%

Fig. 1. e-duk@cja system – number of visits in the period of time from May 18th 2009 to May 18th 2010 [8]

total number of certificates distributed between February 2005 and May 2010 exceeded 73 000 [7].

After Poland had accessed the European Union in May 1st 2004, many young healthcare professionals, including pharmacists, decided to work abroad. Nowadays there is a significant number of Polish pharmacists working in Great Britain, especially in Tesco and Boots community pharmacies. There are also Polish pharmacists who work in community and hospital pharmacies in Ireland, Germany, France and Norway. Some of them treat the stay as a professional experience and perfect pharmaceutical language training but probably consider coming back to Poland. For such people e-learning is the only available tool to gain educational credits and prolong the license in Poland.

Using Google Analytics tool [8] we have recently observed an increase of visits from countries such as the UK, Ireland and Germany (Picture 3). This proves the growing interest of Polish pharmacists working abroad and their participation in the life-long learning process [8].

Pharmaceutical Boards

The table below shows the number of users from each Pharmaceutical Board.

Tab. 1. Registered users of the e-duk@cja platform from different Pharmaceutical Boards

Local Pharmaceutical Board (LPB)	Users
Kraków	1321
Katowice	1286
Lublin	1031
Łódź	926
Wrocław	920
Gdańsk	789
Warszawa	765
Poznań	740
Bydgoszcz	570
Rzeszów	485
Białystok	391
Kielce	286
Bielsko-Biała	267
Częstochowa	249
Szczecin	246
Opole	241
Olsztyn	229
Zielona Góra	209
Koszalin	204
Kalisz	157

e-Courses

The registration to the system requires from pharmacists filling an online application form. After doing it correctly candidates receive from administrators on their mailboxes the password which enables them to log in to the system.After logging in, the user can sign up to a chosen course. Administrators of the platform decide within 7 working days on the acceptance or rejection of a pharmacist to the e-course.The access to courses is regulated by Local Pharmaceutical Chambers. Some of them expect regular payment of Chambers' membership fee to enable the access to educational materials. After taking part in an e-course and passing a final test users obtain certificates from the Centre of Postgraduate Education Faculty of Pharmacy Jagiellonian University Medical College, Krakow.

There are 17 currently available e-courses as listed below in Table 2:

– 14 e-courses for *soft* credits – total 28 credits,
– 3 e-courses for *hard* credits – total 16 credits.

Tab. 2. The overview of courses on the e-learning platform e-duk@cja

No.	Course	Type	Credits
1	Pathophysiology and diagnostics of osteoporosis	Soft	2
2	Pharmacotherapy of non specific gastritis	Soft	2
3	Drug from a computer	Soft	2
4	Prevention and pharmacological treatment of osteoporosis	Soft	2
5	Peptic ulcer disease	Soft	2
6	Efficiency and safety of pharmacotherapy	Soft	2
7	Pharmacotherapy of urinal system's infections	Soft	2
8	Actual possibilities of pharmacotherapy of some neural system diseases	Hard	8
9	Chosen aspects of pharmaceutical care	Soft	2
10	Pharmacogenetics – the influence of genetic factors on the efficiency and safety of pharmacotherapy	Soft	2
11	Drug safety	Soft	2
12	Chosen problems of infectious diseases' therapy	Soft	2
13	Obesity and overweight as a health problem	Hard	4
14	Drug durability	Soft	2
15	The role of interpersonal communication in pharmaceutical care	Soft	2
16	Pharmaceutical care for patients with epilepsy	Soft	2
17	Promotion of health and health prophylaxis in community pharmacies	Hard	4
18	Modern pharmacotherapy of arterial hypertension	Soft	2
19	Progress in pharmacotherapy of metabolic diseases	Hard	10
20	Modern pharmacotherapy and the role of a pharmacist in a treatment of a cardiovascular disease	Soft	2

	Courses withdrawn for further update
	Courses granted with hard credits
	Courses granted with soft credits

Each course finishes with an online test. To pass the test users need to choose at least 60% of correct answers. You can take the online test only once.

Conclusions

Analyzing the dynamic change of the amount of users we predict to have 12.000 pharmacists registered to the system till the end of the year 2010.The creators of the platform are going to add more e-courses, especially the ones granted with *hard* credits. Also, another aim for us is to make the courses more attractive for learners by adding multimedia materials like audio and video files.

We hope to create interactive lectures during which participants and a teacher can see and listen to each others in a virtual classroom. We plan to systematically add courses from various fields of pharmaceutical sciences to give our participants the chance to be trained in different areas.

Last but not least, we always encourage pharmacists to give us a feedback about the e-learning platform we administer. It helps us to improve it and get know expectations of pharmacists involved in a life-long learning process.

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EDUCATIONAL DECISION DIAGRAMS IN BIOMEDICAL AND LIFE SCIENCES – EXPERIENCE GAINED FROM INTRODUCING INTO ANTHROPOLOGY CLASSES

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Abstract: Graphic organizers are visual knowledge representations believed to be helpful in understanding relationships between concepts and processes. The goal of this study was to verify whether replacing a linear textbook chapter with an interactive graphic organizer improved students' reaction, knowledge retention and transfer in an anthropology class. The applied graphic organizer, an educational decision diagram in recognizing human developmental age based on dentition, was created using the Bit Pathways tool. Students were randomly divided into two groups, one using an interactive, web-based graphic organizer, the other using a traditional textbook chapter. After a week students were tested on retaining knowledge and practical skills. No statistically significant differences in learning effect could be observed between the experimental and control group. Nevertheless, the results of students satisfaction survey show that students actually enjoyed the class, appreciated innovation in their learning activities and subjectively assessed the pathway as more interesting and effective than the textbook chapter.
Keywords: Educational Decision Diagrams, Graphic Organizers, Block Diagrams, E-learning

Introduction

Graphic organizers have a long history of usage in education [16,20]. They may be defined as "spatial arrangements of words (or word groups) intended to represent the conceptual organization of text" [19] or as "two-dimensional visual knowledge representations that show relationship among concepts or processes by means of spatial position, connecting lines, and intersecting figures" [16]. The most broad scoped definitions of graphic organizers include several types of diagrams as concept and knowledge maps [3, 4], flowcharts [5, 6], hierarchies, matrices, and many more [19].

Graphic organizers generated by the Bit Pathways application [7] form an extension of the classical flowcharts concept [22] enhanced by the interactivity of the flowchart components. The Bit Pathways flowcharts (called educational pathways) are web-based diagrams which present individual steps and decisions involved in a real-life process. The diagrams, exportable to HTML-format, display in response to selection of the flowchart elements by the learner (via a mouse cursor) a set of attributes' values (attributes taken from predefined templates) linked with the selected element. In addition, to reduce the complexity of flowcharts, the diagrams may be broken down into a set of sub-pathways, which may be viewed in a learner-specified order. The primary application of the Bit Pathways program was to serve as an aid in clinical pathways authoring [7], however, it turned out to be flexible enough to help in creation of graphic organizers also in other than medical fields – e.g. chemistry [1, 9] and statistics [18].

Educational pathways have the potential to be a helpful learning tool from the viewpoint of the Mayer's cognitive theory of multimedia learning and his principles of multimedia design [13]. It is argued that pathways created by the Bit Pathway tool adhere to:

(a) the segmenting principle – the lesson is broken down in user-paced descriptions of individual process steps rather than presented as a continuous unit,
(b) the multimedia principle – pathways have a two di-mensional graphical form, the element descriptions may contain not only words but also pictures,
(c) the signalling principle – the graphical flowchart con-figuration indicates the correct order of steps involved in the described procedure. In addition, values of attributes may be formatted (e.g. bolded) to highlight the most important messages,
(d) the spatial and temporal contiguity principles – the description of the selected element is displayed in a pop-up win-

dow located next to the selected place immediately after activating a flowchart component by a mouse cursor.

There are two substantially different modes of graphic organizers usage: author-provided graphic organizers (i.e. learning-by--viewing) and learner-generated graphic organizers (i.e. learning--by-doing) [19]. Despite the merits of active learning promoted in the learning-by-doing scenario, a recent study undertaken by Stull and Mayer [19] suggests that constructing graphic organizers may increase the cognitive load because of extraneous processing for the diagram authoring and in that way prevent effective learning. In this study we focus on the benefits of introducing author-provided educational pathways into a lesson in anthropology rather than constructing pathways by learners. Research on learner-generated diagrams is carried out in parallel and is reported elsewhere [8, 10]. A second instructional consideration is whether to present graphic organizers before (advance organizers) or after (post organizers) the main learning event. More and Readence meta-analysis suggests the latter option, however, the evidence for that is still not convincing [15]. In our research we decided to present the graphic organizer after an introductory learning module.

Goal of this study was to verify whether replacing a linear textbook chapter with an interactive graphic organizer (in this experiment – an educational pathway) improved students reaction, knowledge retention and transfer in an anthropology class. The experiment was carried out at the University of Physical Education in Wrocław. Topic of the presented edu-cational pathway was determination of the human developmental age. The ability to estimate developmental age of children is important for physical education students, however, it is often neglected by their teachers and coaches. The level of biological development, often different from calendar age, determines psychomotor abilities in children [2, 11, 14]. Lack of this knowledge may lead to overloaded physical exercises at school or in team sports and in turn result in increased risk of injuries and unfair grading of children's efforts. One of basic methods of developmental age estimation is analysis of dentition (dental age) – i.e. determination of the number and type of erupted teeth [12, 21].

Material and methods

Various kinds of educational materials were prepared for the purpose of this study:

– a virtual patient case "Adaś" containing basic knowledge about type and number of primary teeth and permanent teeth (Fig. 1),
– a dentition development pathway (dental pathway) in the form of an educational decision diagram for estimation of dental age, created by using the Bit Pathways application (Fig. 2),
– anthropological textbook chapter about dental age,
– test images showing dentition in children.

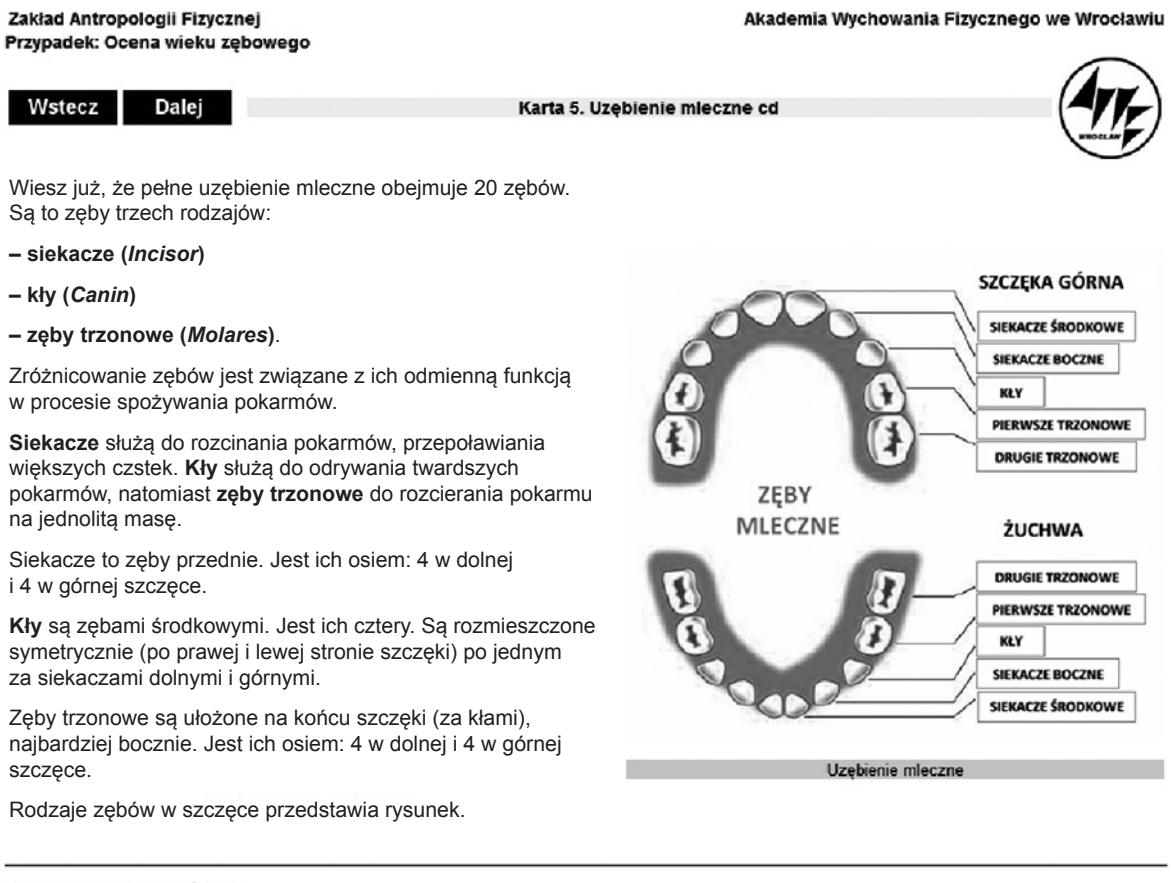

Fig. 1. Virtual patient case "Adaś"

The textbook chapter contained approx. 800 words, 25 schemas (dentition images) and 3 tables. The dental pathway included approx. 2000 words, 11 dentition schemas and was accessible through a standard web browser. The text content and images of the dental pathway were displayed upon selection of the relevant shape with a mouse pointer (Fig. 3).The virtual patient case was a simple web application presenting a set of linearly ordered screen cards showing the progress in teeth growth of a child in a 14 years' period.

All materials were presented in a self-study scenario, in which students were invited to attend a class and asked to learn individually using a computer. None of the students had prior experience with dental age estimation. The scenario in the first 15-20 minutes was the same for all students (N = 30) and involved reading basic information about dentition presented as a computer-based anthropological case (virtual patient). The second part, in which students were randomly divided into two groups, took 20-30 minutes. Members of one group (N = 17) were studying individually a dental pathway, whereas the members of the second group (N = 13) were reading a textbook chapter. Finally, every student got three pictures with dentition in order to practice estimation of the child's developmental age.

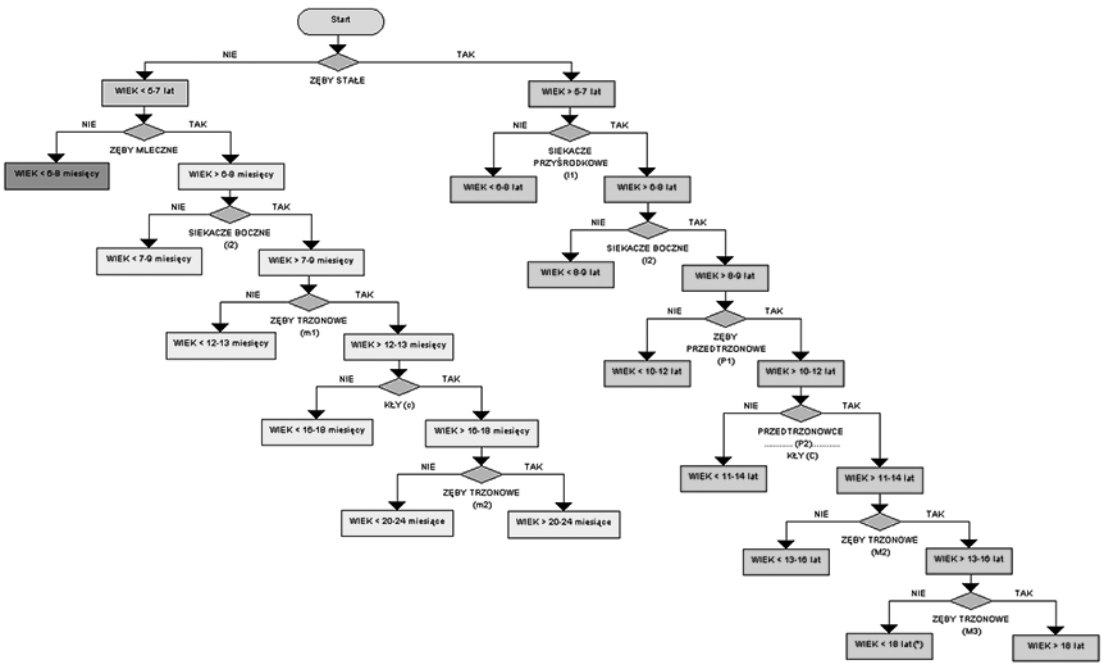

Fig. 2. Overview of the dental pathway

One week after the class students were tested to investigate whether gained knowledge and skills could be retained. Students were not informed in advance about the test. The subjects were given a short knowledge test and five pictures of children teeth to check their practical skills. Students' satisfaction with the learning scenario was measured by a survey consisting of 5-point Likert's scale questions and free-text questions.

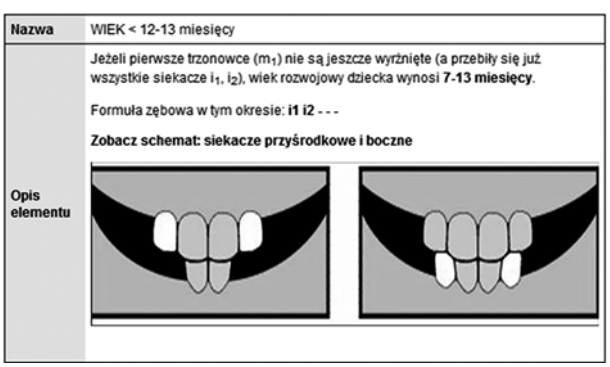

Fig. 3. Pop-up window activated upon selection of a pathway element by a mouse pointer

Obtained data were analyzed using descriptive statistics, significance tests of median difference (Mann-Whitney) and finally, correlations between the study variables were sought (Spearman r).

Results

It was shown that students' basic knowledge (gained during self-study with virtual patient Adaś) was good (Fig. 4).

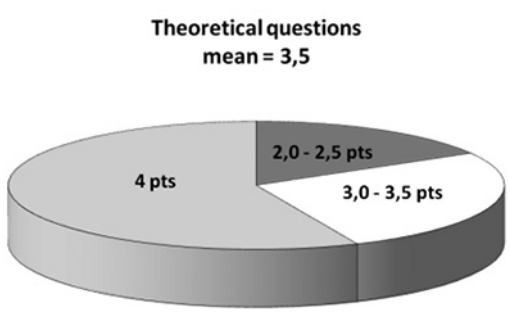

Fig. 4. Students' results in theoretical questions

The average mark in theoretical questions was 3,5 (in a 4-points scale). Students had no problems with text comprehension in the anthropological case description ("*Was content of virtual patient comprehensible?*" average 4,6 in 5-points scale), textbook and developmental pathway (respectively mean 4,0 and 4,5). Most of them agreed that both textbook and pathway were helpful learning resources to learn evaluation of dental age (Fig. 5. average 4,1 in textbook group and 4,4 in pathway, no significant difference ($p = 0,22$)). Developmental pathway was rated as a good tool for self-study (mean 4,2). Positive students' attitude was confirmed by practical test cases (Fig. 6). In addition, the higher grades students had in theoretical part, the better they managed with practical tests ($r = 0,45$; $p < 0,05$). While comparing practical skills of students in both groups, it occurred that there was no significant difference in the retaining effect of self-study between textbook and pathways (Fig. 7). Students learning with dental pathways reached 5,4 points in average, whereas students using anthropological textbook reached 5,8 points (in 10 points scale; $p = 0,66$).

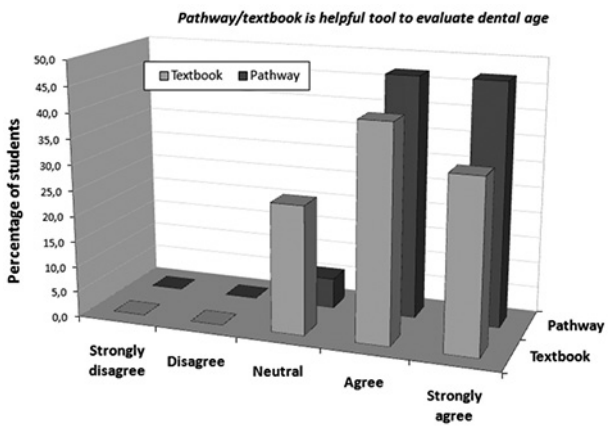

Fig. 5. Comparison of students' opinion on usefulness of the pathway and textbook

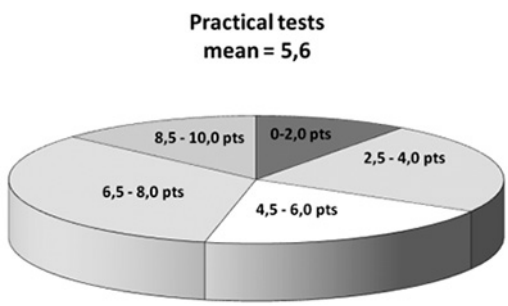

Fig. 6. Students' results in practical tests

Even though both educational material types were found understandable and appropriate for learning, students presented ambivalent attitude to the statement "*I have learned to evaluate dental age*" (mean for textbook 3,5; mean for pathway 3,7 out of 5). It seems that many students are not able to learn practical skills without any help from the teacher's side. They are used to rote learning but do not know how to apply their knowledge in practice.

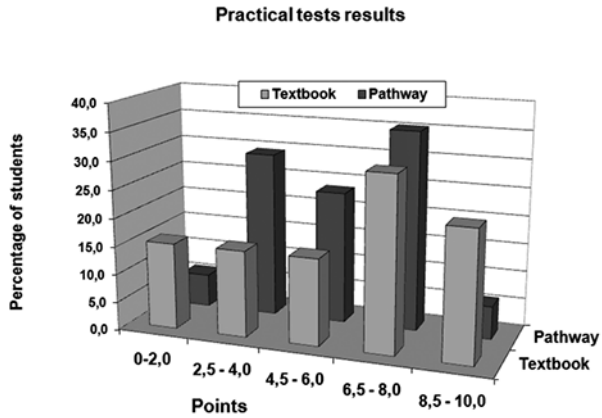

Fig. 7. Comparison of students' in practical tests for the experimental and control group

Many students in free-text questions underlined that the class concerning dental age was interesting and informative for them (mean 4,3 in 5-point scale) and that this was their first encounter with this form of learning (only 3 students stated that they used educational decisions diagrams before). They claimed that they would like to have more lessons like that one in future. They also admitted that learning with computer was more interesting for them (mean value 4,5) and more effective (mean value 3,8) than learning from a textbook. In the free text comments some students stated that Internet access gives them the opportunity to learn anytime, anywhere and to reach interesting information just-in-time. However, there were also opinions supporting learning from traditional textbook rather than using computers. Some students prefer textbooks because they can better concentrate on learning – they perceived computer as a tool for entertainment but not for learning.

Discussion

The obtained good effects in knowledge acquisition of human developmental age based on dentition could be reached by the application of appealing educational tools, such as virtual patients. Students were very interested in the presented case "Adaś" – they were studying eagerly and solved their tasks efficiently. The skills learned by students during self-study scenario were also satisfying regardless the type of applied learning material (textbook or pathway). Students in free-text answers underlined that the dental pathway helped them in systematization of knowledge and memorizing consecutive steps.

Evidence showing effectiveness of different kind of diagrams was summarized in several reviews and meta-analyses (e.g. [3, 16, 20]). Graphic organizers are believed to pre-attentively convey information through spatial configuration of components (a so-called visual argument) and the human ability to discriminate individual components (chunks of information) from each other [20]. A diagram can show at the same time many key concepts helping the learner to build a schema of what is to be learned. Relationships presented in the text are made by graphic organizers very concrete and visible. Spatial arrangement and verbal knowledge are transmitted by separate information-

-processing channels [13,17] and in that way the text, illustrated by graphic organizers, despite containing more information does not necessarily increase the cognitive load of the learner. Because verbal and visuospatial knowledge is believed to be stored in separate but potentially interlinked memory regions, the duality of encoding may provide an additional retrieval path for both kinds of information [16]. Despite these theoretical arguments we failed to demonstrate the superiority of graphic organizers over text description in this experiment. The reasons for that may be various: small sample size, briefness of the knowledge and skills evaluation or specificity of the topic selected as source for the pathway.

Worth mentioning is the fact that students perceived the dental pathway rather as a good reminder of how to evaluate dental age than a resource helpful in learning the algorithm. Students claimed that they evaluated age of children easier and faster having access both to test images and the pathway at the same time. When it came to practical application of the knowledge without access to the pathway, some of the students failed to succeed. Both student groups – using pathways and using textbooks – were not convinced about their own proficiency in recognizing the dental age. On the one hand this observation shows lack of abilities to apply the acquired knowledge in practice, on the other hand it indicates a strong need of a personal contact between students and the teacher, who can emphasize practical aspects of the presented knowledge.

The dental pathway discussed in this paper is just one example of a series of pathways created using the Bit Pathways tool. Other pathways in ontogenesis and sport medicine inform about evaluation of the developmental age on the basis of skull sutures and skeletal ossification procedures or allow gender recognition at different levels of body complexity (genetic gender, gonadal gender, genital gender, somatic and psychical gender). These pathways are still waiting to been verified in practice.

Conclusions

The retaining effect of self-study was more visible in the theoretical part of the experiment than in the practical part. We did not observe significant differences in the level of knowledge and skills between tested learners studying from textbook and those using a dental pathway. Many students appreciate and prefer modern didactic methods accessible by computer, but it had no apparent effect on the learning outcome. We observed also students who gave priority to systematic learning from textbook over innovative techniques.

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THE CRITERIA OF LIFE AND AGING FROM A MOLECULAR VIEWPOINT. THE ROLE OF PROTEIN AGGREGATION IN THE PROCESS OF AGING

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Abstract: Biological knowledge is expanding rapidly, delving deep into nature's mechanisms. However, the essence of life as a molecular process still remains unclear.
Organized and independently operating biological systems are commonly thought to be living. Unfortunately, these characteristics are too general and altogether insufficient to accurately delimit the boundaries of life. The problem of the relation of many primitive biological entities to the living world is still open. The properties of self-dependent biological systems clearly derive from their highly organized automatic nature.
The comparative analysis of genomes of primitive biological organisms seems to be the most promising approach, which may eventually lead to the understanding of life at the molecular level and its definition. The erythrocyte appears to be of particular interest as a model of a living system that is at a boundary. Its biological origin, automatically controlled metabolism, and programmed death sharply defined in time qualify it as the living structure, even though it is completely deprived of a genetic apparatus. However, its membership among living systems seems to be well-founded.
Protein aggregation is one of the common characteristics of aging. It is a consequence of abnormalities of protein structure induced by destructive actions but also by abnormalities of synthesis. Aggregation of membrane proteins probably affects the activity of certain enzymes or transport proteins, which are important as energy providers for aging erythrocytes. After the erythrocyte has passed through the vascular system a given number of times, it is not able to undergo a certain set of indispensable metabolic rearrangements.
A living thing is then a form of animated nature which has the features of independence as a result of automation and possesses its own compatible with nature program of action which is time-limited beforehand.
Keybords: Life, Protein aggregation, Eryptosis, Erythrocyte

In search of a definition of life

Our understanding of nature and its mechanisms improves gradually with increasing knowledge of biology. However, the concept of life as a phenomenon remains ambiguous [1]. It is apparently obvious but still elusive. It is expected that progress in the knowledge of the molecular mechanisms will provide further insights into the issue. Some scientists compare the difficulties relating to the definition of life to those encountered while describing the properties of water or fire until focusing interpretation on the molecular structure [2]. Because of problems in definition life is usually described as a set of signs listed from the viewpoint of metabolism, biochemistry, genetics and thermodynamics. However, this descriptive classification does not explain the complexity of life as a process. When using this definition some evidently inanimate objects are sometimes classified as living organisms and vice versa. Newer definitions combine the concept of life with the genetics. Living things are systems that are capable of self-reproduction, mutation and natural selection [3]. Life is also a self-sustained chemical system capable of undergoing Darwinian evolution [4].

Self-organized and self-sustaining biological systems are classified as living organisms. This definition is too general and does not delimit precisely the boundaries of life. There is still controversy regarding criteria for classifying biological forms, especially subcellular, as living things. This concerns for instance viruses. In contrast to bacteria, viruses do not have independent metabolism and despite their biological origin they usually are

not regarded as living organisms. There are also cybernetic and thermodynamic approaches to describe living things [5].

In thermodynamics, systems in which some processes occur are divided into open and closed systems. Reversible processes occurring in closed systems, not linked with the surroundings, tend to reach an equilibrium, which occurs when the reactions in both directions are taking place at the same velocity. This moment is regarded as the end of the reaction. In the cell this state is attained upon its death. However, life is sustained only by processes that occur spontaneously as a result of maintaining non-equilibrium states. These are states, in which the process is still organized and occurs spontaneously (Fig. 1).

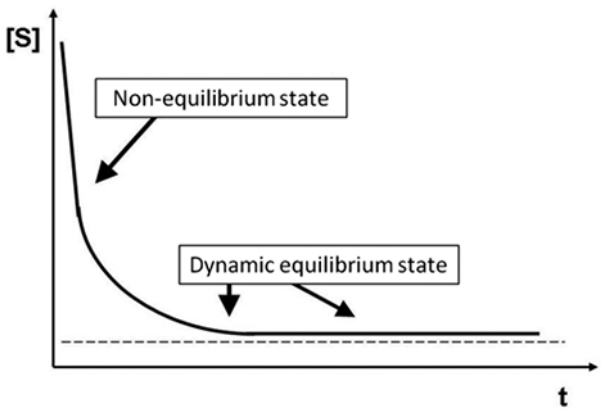

Fig. 1. Reaction kinetics in the model system with non-equilibrium and dynamic equilibrium states indicated by arrows

Maintaining non-equilibrium states is possible only with permanent exchange of the reaction components with the surroundings, that is, in open systems where inflow and outflow of the components maintain the reaction. However, the attainment of the specific, stable level of process-related activity in such systems requires regulation. Only negative feedback auto-regulation may assure stability in the situation where inflow and outflow of the reaction components is dependent on the surroundings, that is, it is prone to oscillation. The omnipresent automaticity in complex biological processes stigmatizes the cells and the organisms and is interpreted as the characteristics of life.

The automaticity of nature

It appears that the most consistent definition of life, which is still valid today, was provided by Claude Bernard in the 19th century, who explained that "the stability of the internal environment is the condition for free life. All of the vital mechanisms, however varied they may be, have always one goal, to maintain the uniformity of the conditions of life in the internal environment" [6]. In our understanding this definition explains also the ultimate goal of cells and living forms. In his definition Claude Bernard introduced also the idea of independence, that is, the independence which the organism has of its external environment. The freedom of action, which increases with the increasing complexity of biological systems, appears to be the most common feature of living forms. Freedom is a measure of the complexity of automatic structures. The decision to initialize a function is made in automatic closed-circuit systems, and the range of freedom increases with the increasing complexity of the system via regulatory coupling. The possibility of making the independent choice is a measure of freedom.

The characteristics and properties of biological forms we refer to as living may be accounted for by their automaticity. However, it is difficult to accept the simple understanding of biological systems as automatic structures. Today, due to technology and especially robotization, we are capable of creating automatons close to living forms. The demarcation line between living things and man-made structures becomes blurred.

However, biological entities are different even from the most wonderful robots in the fact of belonging to the natural world which has its laws and rules stigmatizing individual entities. The differences stem from this very fact. The belonging to the natural world imposes certain rules which are unknown in the sphere of automation and robotization. This results in the occurrence of specific properties. One of these properties is programmed cell death – the process which is fundamental in nature as a whole. It stems from the philosophy of nature, not of the individual, and is a prerequisite for maintaining balance and development in nature. Programmed death is common to all normal (in compliance with the philosophy of nature) biological individuals. It becomes manifest in the determined number of cell divisions, aging and apoptosis. The setting of the final stage, i.e. death, in a life process program requires the inclusion of a time factor and clock mechanism. Process periodicity, including first of all the circadian cycle, is an integral part of living organisms [7, 8]. The individuals without the mechanism of death in their cycle (for instance immortal neoplastic cells) are regarded as a foreign and destructive element. Immortal and non-aging cells do not adhere to a living thing program. They are not consistent with the definition of biological life because they do not possess the program of death. In this respect the definition of life must be conventional assuming that nature itself determines what is alive. While analyzing the characteristics of biological forms on the border between the animate and inanimate world, one may become aware of the fact how far the inevitability of death is binding in the natural world.

The erythrocyte as a model system for determining the limits of biological life

Analysis and comparison of the genome in primitive organisms will really soon show which genes and functions are responsible for what we refer to as life. As expected, this should be a repetitive set of genes that determines automatic maintenance of internal stability. However, this condition can be fulfilled in the system devoid of genes for example in the erythrocyte. It has a biological nature because of its origin, but it cannot reproduce in its functionally mature form. As it originates from a fully developed cell, it may serve as a convenient test system.

The erythrocyte (normocyte) is the final developmental form of an erythrone. It originates in the nucleated cell (acidophilic erythroblast) from which the nucleus is expulsed dur-

ing the process of differentiation to the mature blood cell. An anucleated reticulocyte containing reticular accumulations of remnant RNA, ribosomes and mitochondria is an intermediate stage. As compared with nucleated cells whose proteome has about 20 000 – 30 000 proteins, erythrocytes contain about 350 various membrane proteins and 250 soluble cytoplasmic proteins [9, 10].

Erythrocyte life-span, measured using cohort methods (incorporation of isotope into newly synthesized blood cells) and random-label methods is about 120 days [11]. Erythrocytes preserve automatic metabolic equilibrium (steady state) to assure indispensable appropriateness of ionic milieu and oxidation-reduction potential. The abrupt, not smooth termination of life of the red blood cell implies the existence of a mechanism which defines the end of the function. The maintenance of internal stability despite dramatic changes in the external environment having impact on the cell in the organism indicates that automaticity is its reliable feature.

Erythrocytes perform hard work by counteracting environmental conditions while traveling between the respiratory system and tissues. Human red blood cells every minute on average enter pulmonary capillaries where they unload carbon dioxide and protons (the Haldane effect). The oxygen-rich environment of the lung and transport of oxygen bound to hemoglobin increase exposure to oxidative stress [12]. Hemoglobin autooxidation reactions are the main source of active superoxide anion radicals in the erythrocyte [13]. In these reactions oxyHb transforms into MetHb releasing superoxide anion radical. It appears that in oxyhemoglobin Hb-Fe^{++} - O_2 is in equilibrium with Hb-Fe^{+++} - O_2^-. The latter form may undergo spontaneous dissociation. About 3% of oxyhemoglobin per day is dissociated in this way under physiological conditions. Superoxide anion radical may undergo further reaction to obtain active derivatives (Fig. 2). The activity of mainly enzymatic antioxidant systems decreases the amount of metHb to about 1% [14].

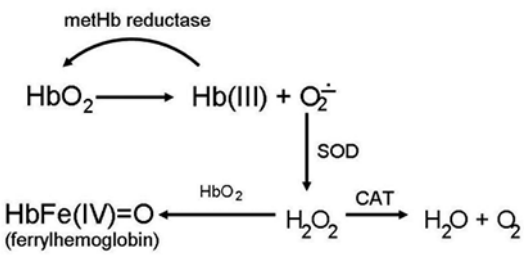

Fig. 2. The cascade of hemoglobin autooxidative reactions. The abbreviations used are: SOD, superoxide dismutase; CAT, catalase

Additionally, certain xenobiotics and infections increase exposure to reactive oxygen species. They produce hydrogen peroxide, which in the presence of reduced glutathione is neutralized by glutathione peroxidase. One of the serious consequences of the action of oxidative factors on the erythrocyte is the formation of denaturated hemoglobin deposits, so-called Heinz bodies [11]. Hemichrome and hemin, generated during hemoglobin denaturation, may bind to the erythrocyte cell membrane, thus causing its damage. Glutathione is the main low molecular weight antioxidant whose concentration in the erythrocyte is high (about 2 mM). This tripeptide is synthesized in the presence of ATP without ribosomes.

The erythrocyte maintains the normal function despite cyclic temperature differences (28°C in the pulmonary tissue and close to 40°C in the liver and working muscles). High osmolality in kidney medulla additionally exposes erythrocytes to osmotic stress. Erythrocyte diameter is larger than capillary diameter, causing friction and deformation during the passage [15].

In the body, the erythrocyte cytoskeleton is most of the time forced to undergo structural rearrangements. The work counteracting these processes requires energy (ATP and reduced pyridine derivatives). In humans, ATP in erythrocytes is formed via the fundamental metabolic pathway, i.e. glycolysis (Fig. 3). In the absence of mitochondria in erythrocytes glycolysis produces lactate, which is removed from the cells.

The passage through metabolically active tissues, i.e. the environment with high acid content (for instance skeletal muscles), alters the rate of passage of the intermediates through the metabolic pathways in erythrocytes. Regulation of glycolysis in erythrocytes is especially sensitive to changes in acidity. Reduced pH decreases the activity of phosphofructo-kinase-1 and other important allosteric enzymes: pyruvate kinase and hexokinase. Hydrogen ions inhibit the reaction catalyzed by bisphosphoglyceratemutase and stimulate phosphatase activity of this bifunctional enzyme. Therefore, severe acidosis reduces the concentration of 2,3-BPG and ATP in erythrocytes and increases the affinity of hemoglobin for oxygen [11,16]. For this reason, red cell glycolysis is very H^+ sensitive, being stimulated by a rise in pH. The available evidence suggests that the pH is the primary controlling factor of erythrocyte metabolism.

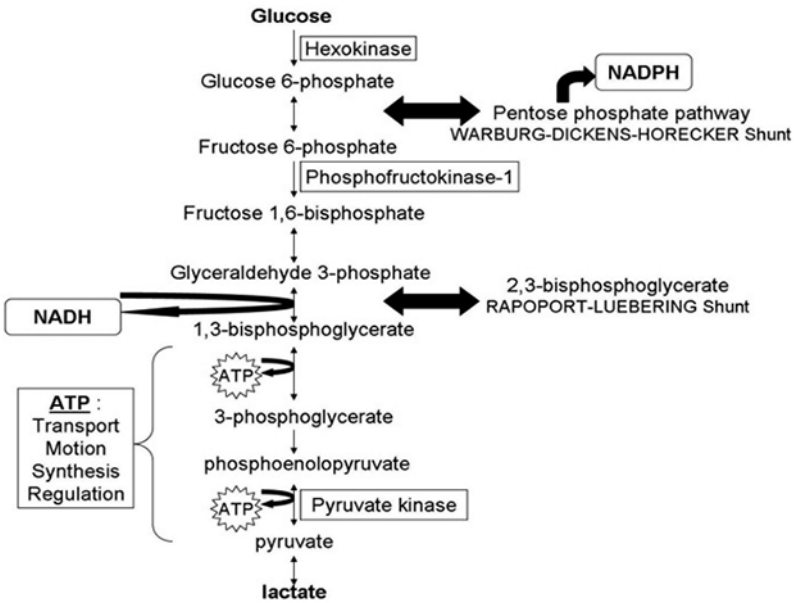

Fig. 3. The basic metabolic pathways in erythrocytes.
In boxes, regulatory enzymes whose activity decreases in the presence of high acid content

Cytoplasmic pH was found to be slightly lower in old blood cells than in the young ones [17]. However, reduction in enzymatic activity in aging erythrocytes followed by reduced ability to synthesize ATP was not confirmed. It appears then that it is not the main reason for blood cell death [18,19]. Reduced activity of hexokinase, glucose-6-phosphate dehydrogenase and pyruvate kinase in reticulocytes persists only for several days after the appearance of erythrocytes in the circulation, however, it remains unchanged afterwards. The only exception is permanent reduction in the activity of pyrimidine 5`-nucleotidase and AMP deaminase [11]. However, reduced activity of these enzymes may be a part of the blood cell program, because DNA is almost totally removed in the denucleation process, whereas RNA which is useless for the remaining stages of erythrocyte's life undergoes enzymatic degradation. Especially CMP and UMP do not play any further metabolically important role in the erythrocyte, therefore they must be degraded to nucleosides which undergo diffusion [21].

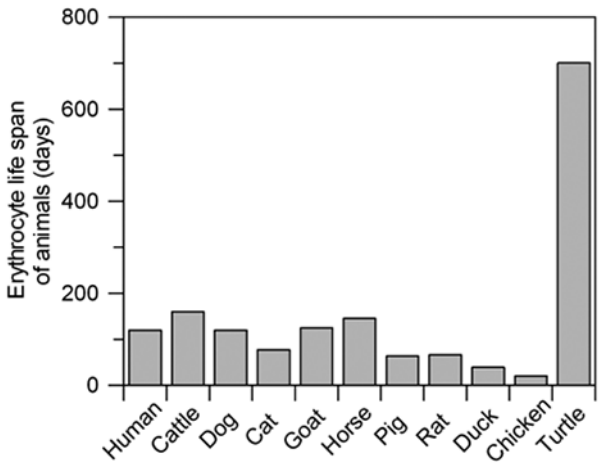

Fig. 4. Average erythrocyte life span in various animal species

Eryptosis – death program of erythrocytes

The erythrocyte rigorously obeys imposed rules within the relatively narrow range of freedom. As a matter of fact the erythrocyte could be viewed as a biological robot which may not necessarily be alive. However, its determined life span is a feature of living systems. The relatively abrupt limit to life, which is species specific, indicates that erythrocyte death is not a simple result of more and more injuries, but of taking into account the internal clock and death mechanism in its program (Fig. 4).

Under these circumstances it is necessary to replace old red blood cells with the young ones. Hemolysis of erythrocytes must be and is in fact controlled. Intravascular hemolysis causing the release of hemoglobin in approximately 200 billion erythrocytes daily would overwhelm the compensatory mechanisms of the body. Therefore, this process is programmed and controlled, similar to apoptosis [12,15]. Only approximately 10% of red blood cells is destroyed intravascularly [11]. Red blood cells are devoid of a nucleus and mitochondria. For this reason there are no cellular organelles that are critical for apoptosis in the traditional sense. However, certain morpho-logical features such as cell shrinkage as a consequence of intracellular fluid loss and characteristic blebbing of the plasma membrane can be found in erythrocytes. Analogically to apoptosis, increased concentrations of calcium ions have also been found in dying erythrocytes. This in turn activates calcium-dependent potassium channel (Gardos

channel) and leads to potassium loss. In order to differentiate this process from apoptosis in nucleated cells, it is referred to as eryptosis. Evidence shows that prostaglandins are also involved in the regulation of erythrocyte death [22]. Phosphatidylserine in the external layer of the cell membrane (being also the feature of nucleated cells undergoing apoptosis) is recognised by macrophages [23]. It is a result of limiting the quantity of ATP available to ATP-dependent flippase, the protein which is responsible for transport of this phospholipid to the internal cell membrane. Additionally, abrupt increase in the concentration of calcium ions during eryptosis leads to activation of scramblase, an enzymatic protein necessary for phosphatidylserine externalization [24]. All these changes enable phagocytes to recognize aging erythrocytes. The above described processes lead to removal of red blood cells from the vascular lumen.

Programmed erythrocyte death allows for control. The breakdown of red blood cells is extravascular and occurs in the monocyte-macrophage system of the spleen, the liver and the bone marrow. Intravascular hemolysis is unfavorable and it may occur in the course of hemolytic diseases, for instance transfusion of incompatible blood in the AB0 or Rh system or in the course of a rare disease paroxysmal nocturnal hemoglobinuria [11]. Therefore, the programmed alteration in cell surface structure is the body's strategy enabling the phagocytes to recognize such blood cells.

Erythrocyte life span is also dependent on osmotic and oxidative stress. Death of erythrocytes is a complex programmed metabolic process. Only 0.06-0.4% of red blood cells, irrespective of their age, are destroyed accidentally, in a non-programmed manner [11]. Interestingly, erythropoietin has been found to increase erythrocyte life span. The protective effects of erythropoietin are observed in dialysis patients when the number of cells exposing phosphatedylserine (which is a signal for cell removal) decreases several hours after hormone injection. It appears then that in exceptional cases the programmed erythrocyte life span can be modified by a signal command [12, 25].

According to the approved criteria erythrocytes, unlike viruses, are living things. They are automatons with a naturally determined life span.

The role of clock machinery in programmed erythrocyte death

The mechanism of aging and dying in nucleated cells is believed to be hidden in the mechanism for telomere length regulation. It must be a different mechanism in case of erythrocytes. The cell membrane is a determinant of life span. Turnover in living organisms and storage of erythrocyte concentrate are associated with gradual loss of membrane fragments [26]. Biologically determined less stable fragments of phospholipid membrane are probably lost [27]. The process is more pronounced in an acid milieu and at elevated (tissue) temperature [28]. This in turn reduces surface area for gas exchange and leads to the loss of proteins anchored in microvesicles shed by erythrocytes. The process decreases also the ability of red blood cells to deform. It is associated with the increased internal viscosity of the erythrocyte and alterations in red blood cell membrane properties. For this reason the return to its original shape during passage through tissues is slower than usually. Red blood cells become spherical. The increased viscosity of old blood cells has been used for separation of cells [29, 30].

After the erythrocyte has passed through the vascular system a given number of times, it is not able to undergo a certain set of indispensable metabolic rearrangements in its sojourn in the lung or peripheral tissues.

Repeated reduction in red cell surface area and volume reaches the limit beyond which the trigger process is initiated to induce changes in the structure of the erythrocyte. It serves as one of the signals that allow macrophages to recognise aged cells. With the loss of cell membrane integrity, erythrocytes also loose various substances that protect them from being recognized by the reticuloendothelial system in the liver and the spleen. The formation of microvesicles with low protein content favors the increase in concentration of certain proteins in the membrane and increases the risk of their aggregation. Such protein conglomerates may bind immunoglobulins, which is an additional signal for phagocytes. Evidence shows that the number of immunoglobulin-coated erythrocytes increases with advancing age [31]. However, it does not appear to be the fundamental mechanism for recognizing old cells – erythrocytes in patients with agammaglobulinemia (inherited lack of immunoglobulins) do not have a longer life span at all.

The role of protein aggregation in programmed erythrocyte death

Aggregation of membrane proteins probably affects the activity of certain enzymes or transport proteins which are important as energy providers, for instance aggregation of band 3 protein (AE1) in aging erythrocytes. It is the most abundant protein of the erythrocyte membrane (almost one million protein molecules in a single red blood cell), accounting for 25% of integral membrane proteins and occupying 10% of red blood cell surface area. This protein, similar to the membrane potential, is regarded as the main factor determining the shape attained by the erythrocyte. Band 3 protein conformational changes induce the outflow of ions and the inflow of anions across the membrane. Its inactivation as a result of aggregation leads to failure to maintain ionic equilibrium in the cell.

The conformational changes of the band 3 protein and related probable enzymatic processing result in decreased activity of certain glycolytic enzymes which bind to the protein intracellular fragment. This process inactivates the following glycolytic enzymes: phosphofructokinase, 3-phosphoglycerol aldehyde dehydrogenase and aldolase. Regulation of complex formation involves also phosphorylation and dephosphorylation of band 3 protein N-terminus with the process being dependent on erythrocyte oxidoreduction potential [11, 32, 33].

Apart from loss of certain proteins that are important for maintaining erythrocyte function, the decrease in cell surface leads also to changes in its shape and spatial arrangement of cytoskeletal proteins (they are attached to band 3 protein).

Impairment of any element in internally coupled metabolic reactions results in deregulation which may independently lead to death. It has been proved in blood diseases which shorten blood cell life span. They arise from defects in metabolic path-

ways (hemolytic anemia due to deficiency or reduced activity of glycolytic enzymes, defects of the pentose phosphate pathway, and anemia resulting from alterations in the cytoskeleton and defects in integral membrane proteins).

Protein aggregation as an element of aging process

Protein aggregation is one of the common characteristics of aging. It is a consequence of abnormalities of protein structure induced by destructive actions (free radicals, mechanical impact, temperature) but also by abnormalities of synthesis, mainly due to mutation. The cell defends itself by destroying abnormally structured proteins, determining short life span for unstable proteins, altering the rate of synthesis and the rate of protein elimination. Metabolism gradually slows down with aging and the amount of abnormally structured proteins increases which in consequence accelerates the rate of aggregation [34, 35]. A constellation of pathological processes that ensue are natural companions of aging.

Discussion

The mechanism that determines red blood cell death is erythrocyte specific and underlies the universal character of dying in nature. As a consequence, the definition of life appears to refer to automation that provides biological structures with the features of life, but belonging to the living world is determined by the programmed duration of functioning terminated by death.

A living thing is then a form of animated nature, which has the features of independence as a result of automation and possesses its own compatible with nature program of action which is time-limited beforehand.

In accordance with this definition living things are those natural creatures which form the integral part of nature promoting its development. It means that Nature creates things defined as living providing them with the features of independence and simultaneously limiting their time of function. Only combined conditions determine the consistency with animated nature and provide a basis for interpreting the forms of nature as living. In this sense immortal cells are not consistent with nature, and they may be regarded as automatic structures similar to man-made robots. In turn, viruses lacking independence may correspond to cell destroying venoms and toxins.

Among the characteristics of living things one feature appears universal and it is the presence of clock mechanisms. An oscillator is a system that includes a negative feedback loop, which is coupled with a positive feedback loop to obtain appropriate signal characteristics [7, 36]. They usually involve a specific process serving as the basis of time but as a result of cooperation they cover much wider range of functions.The universality of variable frequency oscillators, which are encountered in almost all cells (including bacterial cells), appears to emphasize the real need for self-sustained rhythmic activity. The frequency of oscillations ranges from one cycle per second to one cycle per day or even per year. Humans have circadian rhythms coordinated by one master clock being superior to peripheral oscillators. The circadian rhythm in humans developed in line with the evolution of life on Earth in the light-dark cycle. The role of the circadian rhythm may be to anticipate changes in individual activity related to the onset of daylight. For this reason the master clock in humans (the suprachiasmatic nucleus - SCN) is linked to the retina through neuronal communication [6].

Variable frequency oscillators in cells (regulation of glycolytic enzyme activity, changes in the concentration of calcium ions and others) perform a different function. They include a regulatory feedback loop between DNA and protein. The function of the master clock in humans is regulated by cyclic changes in the activity of the transcription factors CLOCK and BMAL-1 acting as transcriptional repressor (upon Per and Cry proteins).

The role of this phenomenon is not clear yet. Evidence shows that there is a relationship between the presence of oscillators and various individual processes in the cell. However, the universality of this phenomenon may imply its general and fundamental role of maintaining an active form of life processes, which being automatically controlled have a natural tendency toward minimizing their activity upon reaching the programmed level.

Further studies are warranted to answer the question whether the presence of oscillators is a characteristic feature and a prerequisite for life and whether it may be considered as one of its criteria.

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STEERING OF THE PROCESS OF PENETRATION OF CHEMICAL SUBSTANCES INTO BIOSYSTEM STRUCTURE

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Abstract: The analysis of literature relating questions of processes of penetration through organic membranes, such as skin tissue or osseous of the substance, both treating as well as harmful, requires further investigations, because the present descriptions are insufficient for the new measuring challenges connected with applied contemporarily substances in chemical as well as in mechanical formula. This problem is particularly important in the selection of suitable active substances which will accelerate or delay in a well-known manner the process of penetration of the proper medicine through organic membranes into biosystem structure, subjected for specific medical proceeding for the achievement of a declared aim. The penetration through skin tissue of substance of type as the caffeine, theophylline stimulating the central nervous system can be an example of use for deceleration of process of fatigue or the enlargement of durability and the physical resistance, as well as the upward movement of vigilance or the man's cognitive abilities. Penetration in osseous tissue of medicine from implant is another example where it is delivered according to constructed script of medicine interaction. Acquaintance of kinetics of process penetration in both different medical situations will permit to qualify parameters for steering of this phenomenon. It creates then the possibilities for study of new constructions, as for instance new generation of sensors applied in surgical navigation in compiled operations of removing the cancer cells of head or the neck for assurance of the local treatment. The review of literature shows that processes of penetration have been described mainly in the aim of the diffusive mechanism, but a closer analysis of the question discloses that is somehow a simplification of the problem.

Author proposed the new formulation of question of penetration of medicine from composite implant, basing on statistical estimation of the process of transportation captured in suitable variation method in material engineering, but he also sees the medical uses of this method. The analysis of control of process is determinant in support about shape of Boltzmann's entropy, here approximated in beta distribution in form as $E\,(E_{max} - E)$, where E marks the sum of kinetic energy and the potential of penetrating element. Entropy is considered as a Hamiltonian of discussed variation process, in the form of following integral $\int_0^t H(x(t), \dot{x}(t), \ldots)dt$. The suitable variation process in the following form $\delta \int_0^t H(x(t), \dot{x}(t), \ldots dt = 0$ allows to find and trace the optimum trajectory of transportation of elementary entities into the biological system on basis of an approximation of interaction potentials between transferred medicine and the transferring medium. Euler's equation, which response for the mention variation process in an exemplified formula as $\frac{\partial}{\partial x_i}(E - E^2) - \frac{d}{dt}\frac{\partial}{\partial \dot{x}_i}(E - E^2) = 0$

creates these optimum trajectories of the transportation process of penetration. To obtain the progress in closely local method of medical treatment, one should analyze some investigative procedures, what already author has executed in the individual cases. The test of better control of process penetration is creating the proposed solution and the possibility of steering of this process. The control depends on obtaining expected progress in medical treatment as a result of the working of well-known parameters in the course of process, as well as properties of pharmacological substances, having needed potentials activity usually described by pharmacologists, in this case. It seems that proposed new approach allows for a better diagnosis of process penetration, because it enriches the hitherto existing formula of diffusive analyses, as the analyzed processes by the aim of mathematical apparatus were not always diffusive. This new proposal is also a challenge for pharmaceutical industry to undertake the cooperation.

Keywords: progress of medicine, steering of the processes, analysis of medicine penetration

Introduction

The skin tissue is an effectively operating barrier, separating organism from surroundings, which has been formed by millennia of evolutionary development, and it is the organ of senses which contact us with the environment. It prevents the uncontrolled flow of different media from and to organism, from one side, on the other hand, however, the contact with environment is absolutely indispensable for the functioning of the organism. As the largest external organ of human substance, with the surface of about $2m^2$ and the thickness from 0,1 to 5mm average, it protects before the microbes invasion, it assures gas exchange, by skin we loss the excess of warmth created by exercise, it is a receptor of temperature as well as moisture of surroundings, responsible for energetistic equilibrium of organism. Due to the kind of potential functions it fulfils, the skin is also perceived as a semi-conductive barrier or as the diaphragm, through which it can diffuse xenobiotics harmful for health from the environment into interior of organism. However, it can also be helpful on passing many healing substances, which easily diffuse into biosystem by porous tissue. Therefore, this natural diaphragm has been under the interest of many industrial, pharmaceutical and cosmetic companies, textile industry, economic chemistry as well as military industry thorough the world [1].

This problem is particularly important in the selection of suitable active substances which will accelerate or delay, in a well-known manner, the process of penetration of suitable medicine through organic diaphragm into structure which undergoes of medical treatment for achievement of the expected aim. The penetration through skin tissue of substance of type as the caffeine, theophylline stimulating the central nervous system can be an example of use for deceleration of process of fatigue or the enlargement of durability and the physical resistance as well as the upward movement of vigilance or the man's cognitive abilities [2]. Penetration in osseous tissue of medicine from implant is another example where it is delivered according to constructed script of medicine interaction [3]. Acquaintance of the kinetics of process penetration in both different medical situations will permit to qualify parameters for steering of this phenomenon. It creates then the possibilities for study of new constructions, as for instance new generation of sensors applied in surgical navigation in compiled operations of removing the cancer cells of head or the neck for assurance of the local treatment.

The review of literature shows that processes of penetration have been described mainly by the aim of the diffusive mechanism, but a closer analysis of the question discloses that is somehow a simplification of the problem [4], [5], [6].

The authors usually accept one simplest pattern, where they study the penetration through natural diaphragm as well as the artificial placed in Franz diffusive cell. Supposing the steady state of penetrating process, they record the quantity of xenobiotics passing in time and receive the stream of penetration of given substances from inclinations of straight line in μg/cm2/h or accumulated concentration after 24h concentration, Q24 (μg/cm2). The linear range of resultant data is looking for cases where steady streams occur, but there is a need for an estimation of calculation in other cases which brings large uncertainties of measurements. The reason is that in fact the structure of diaphragm is not uniform.

In reaction of penetrating mass of an implant into osseous tissue, the variation formulation has given the solution as a differential equation describing the speed of penetration of mass of an implant.

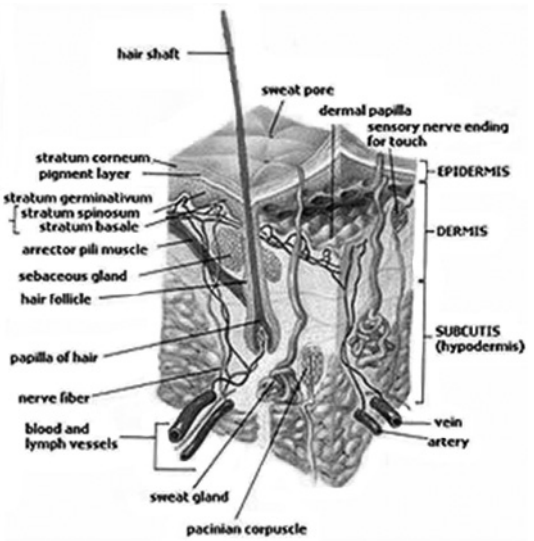

Fig. 1. Cross-section of human skin (from Wikipedia)

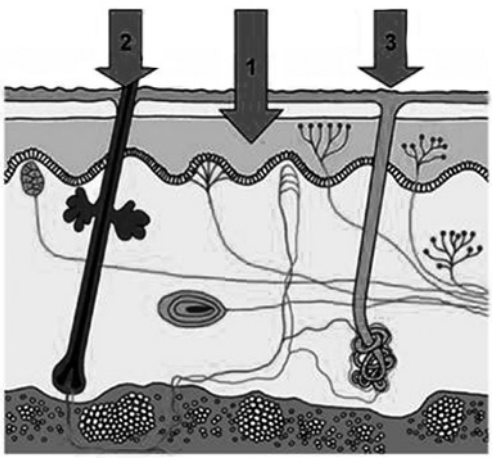

Fig. 2. Possible pathways for and penetrant this cross the skin barrier. (1) across the intact horny layer, (2) through the hair follicles with the associated sebaceous glands, or (3) via the sweat glands [7]

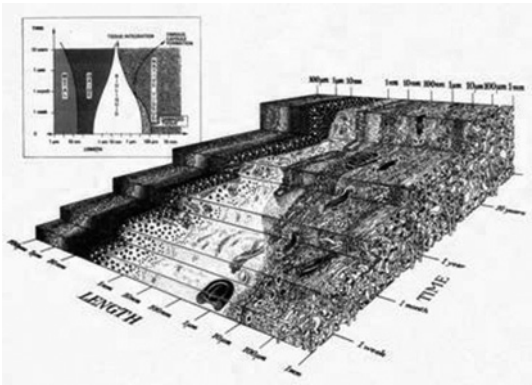

Fig. 3. Dynamic behavior of the interface between implant (left) and bauds tissue (right) [8]

Investigative approach should, however, take into account the compiled structure of skin tissue (Fig. 1). The skin consists from three main layers. Epidermis is the most external protective skin layer, the next one is dermis as a median vascularized layer and the third an internal hypodermis consisting from a fatty tissue layer and connective tissue. The skin contains also hair follicle and bulbs as well as sebaceous and sweat glands. The skin is covered by a horny layer called stratum corneum. Such stratified structure implies the different ways of penetration of xenobiotics, from a straight line of diffusion by skin to proper skin, to different way of transportation through glands channels or hair follicle (Fig. 2). External stratum corneum is built from several dry layers of keratynocites and due to this is a natural, most difficult, obstacle for penetration of ksenobiotics. Also the mechanism of their transportation is difficult to predict. It is assumed that in this layer the process of penetration of chemicals into organism depends on passive penetration by a sequence of layers of cells. Next, numerous metabolic conversions as well as xenobiotic biotransformation take place in the internal layer of epidermis. Any activating of this layer will cause a different course of penetration of different xenobiotics and it is a challenge for an the cosmetic, pharmaceutical and military industry investigators. The hydration of the stratum corneum as well as the change of pH of skin by different solutions or removal lipids from a skin causes a change in its structure, which results in a considerable growth of penetrability of skin for an individual xenobiotics. A different use of various and passive xenobiotics will cause an increase of the resistance of the skin against different toxic substances. The skin penetration process depends on time, but penetration by a stratum corneum is the limiting factor for the speed of the whole process. As the result, the duration of the exposure to xenobiotics is the most essential factor in this case. Moreover, it means also that the quick termination of the contact with xenobiotics, by washing or wiping it off, is the most important action.

In order to achieve progress in close local treatment methods, one should again analyze the investigative procedures. The The author, considering problems of pharmacokinetics in global treatment, proposed the methodology of analysis of local treatment including active treating substances in implants, showing that the program of steering is the beginning of an important medical challenge for chemical laboratories and pharmaceutical industry [9].

Local treatment is also a challenge for material engineering, in the range of construction of new composites with active structures including a new generation of medicines characterized by a selective memory for treatment of a specific organ related with its disfunction and a definition of the individual of sickness (Fig. 3). For instant, the demand that the medical impulse should come from the implant containing a specific medicine has a soliton characterization which is particularly valuable for patient treatment, forces a further analysis of the question.

The new composites are also a challenge for material engineering in range of construction in regard of active structures, including with selective memory the new generation of medicine implants of treatment of concrete organ, related with its disfunction and the given individual sickness.

The essence of the proposition for solution of this problem is the test for a better control of process penetration and the possibility of steering of that process, realized on the base of statistical estimation of the transportation process, formulated in a suitable variation method basing on variation calculation which is well-known in literature [10], [11], [12].

Main result

The diffusive descriptions differ fundamentally from descriptions with approach got by the help of variation calculations. Skipping the only fact of paradoxical unrestricted speeds of a diffusive impulse as well as non dynamic irreversibility, we receive the thickness field of a medium or temperature in result. However, the essence of a variation approach is the question of finding of the best trajectories of the guided impulse, for example the treating one, and of course, the steering of such optimum impulse. This kind of solution is the most coherent with the medical methodology of the treatment of an area, which makes up the focus of disease.

Let us recall the essence of an arithmetic base approach to the problem of getting such optimum useful structures also in medicine. The formula of getting the optimum structures is the problem of finding the best geometries for functional called Hamiltonian of type $\int Hdt = optimum$, where H – Hamiltonian, is in fact the regulating phenomenon function.

The measure of an order in a random process will be such a function in our case. Boltzmann's entropy $f(x,t)lnf(x,t)$ defining the most probable existing state, where elementary particles have positions in points (x,t), where $f(x,t)$ it is the probability density of finding particles in point (x,t), and $f(x, t) = Const\, e^{-E(xt)}$ where $E(x,t)$ is the energy of an elementary particle in that point.

$$E(x, t) = E_{kin}(x, t) + E_{pot}(x, t)$$

$E_{kin} = \frac{\dot{x}^2}{2}$ – kinetic energy, E_{pot} – potential energy.

Let us decide on consideration of independent potentials from time (we will discuss the stationary structures).

Developing the entropy in Taylor's sequence and taking two words of sequence, we can get statistical Hamiltonian in distribution of $beta\ H = E(1 - E)$. Taking the maximum energy as one here, we agree on arbitrary units.

Beta distribution brings closer well-known Gauss's distribution for a definite value of expected random variable here, but

it has zero probability of possession of negative energies as well as larger then maximum (beyond range (0,1)). According to Author's opinion, it is profitable from an ideological point of view, and therefore for so definite "Hamiltonian" in the form as $E(1-E)$ we will look for the suitable optimum structures, in other words, the solutions for a following question:

$$\delta \int_o^t E(1-E)dt = 0$$

where $E(x, t) = \frac{\dot{x}^2}{2} + \varphi(x)$.

The formal problems lead to Euler's equations, well-known in the literature. We will mention only this one dimensional equation here, to avoid compiled mathematical formulas, as follows:

$$\frac{\partial H}{\partial x} - \frac{d}{dt}\frac{\partial H}{\partial \dot{x}} = 0$$

Now let us come into discussion of the most important question from an application point of view, concerning the analysis of potentials decisive about steering of the treatment impulse in the area of therapy procedure. We will begin from discussing the matter of the potential in a sense of Laplace's notification:

$$\Delta\varphi = 0 \quad \text{and} \quad div\nabla\varphi = 0$$

This formula means that there is a lack of sources generating the force, because the force (for example: a force of medicine inte- raction) is in form as $F = -\nabla\varphi$ in every point of a considered area of the medicine.

However, we have to steer with an impulse, so let us write the potential as a following sum:

$$\psi = \varphi + \varphi_1$$

where from: $\Delta\psi = \Delta\varphi_1$

Let's mark $\Delta\varphi_1 = f(x, t)$ where $f(x,t)$ is a source of a force steering with impulse. Hence, we have to solve an equation:

$$\Delta\psi = f(x, t)$$

The chemical reaction of medicine in a place of contagion, and even an external steering of the field of reaction such as electromagnetic as well as mechanical or temperature, can be sources generating these forces.

The obtained equation is not an Laplace's equation but a Poisson's one. The solutions of equation are well-known, but inhomogeneity in equation $f(x,t)$ is unknown and there are only few works devoted to this subject.

The author's work is the proposal of analyses of these procedures, which we can accelerate with analysis of influence on medicine content. The medicine itself, of course, carries on a definite potential, however, it is not known in fact, when reacting in chemically medicine compound in treated area. This potential can be stable if the strength of medicine does not undergo change, or is suitably labile if medicine decreases or causes a prolonged action when is surrounded with isolating substances. There are many possibilities for required solution.

Let us consider the case of one dimensional transportation of medicine into the treated medium, assuming the monotonous decrease of medicine on the way of treatment in following description: $\varphi(x) = A - Ax$

We have got therefore a following problem:

$$\delta \int_o^T [\dot{x}^2 + 1 - x][1 - \dot{x}^2 - 1 - x]\, dt = 0$$

that is the equation (we accepted $A = 1$ for facilitation of calculation), where:

$$H(x, \dot{x}) = \dot{x}^4 - 2x\dot{x} + \dot{x}^2 + x^2$$

skipping coefficient ½ also for omission of needless arithmetic difficulties.

Writing out an Euler's equation for introduced problem we have got:

$$\ddot{x}\,[6\dot{x}^2 + 2x - 1] + 3\dot{x}^2 - x = 0$$

It is a non-linear differential equation of the second order, which the optimum solution of a function $f(x,t)$ which creates the best trajectory.

Equation is unusually difficult to solve. There is a necessity of the numeric approach for that. It is visible, however, that for weakening potential of medicine the shortest healing trajectory will be a compiled function of time.

Let us notice, however, that the found trajectory is the best for process of medicine treatment. Technician treatment operator has to take the care about the presence of the suitable portion of medicine on this trajectory. The different ways have been indicated already in this text. So just thw technician of treatment has to look for the way of an agreement of a treatment process with transportation of medicine on the best trajectory.

Summary and conclusions

The question of penetration of the xenobiotics into the structure of a biosystem such as the tissue of skin or osseous, in point of view of the pharmacological treatment as well as toxic interaction, can be described both by a diffusive model and the proposed new variation approach.

The field of density of diffusive medium or temperature is a solution in this first model, however, the best trajectory of healing or toxic impulses is always obtained in variation model.

In diffusive model we observe the retention of the chemical substance on both sides of an organic membrane, however, it is possible to observe the retention of penetrating substances in every point of this membrane in a new variation considerations.

It seems that the proposed new approach allows for better steering and controlling of resources of such optimal impulse and for obtaining the expected progress in treatment and it enriches the hitherto existing formula of diffusive analyses, because the analyzed processes by the aim of mathematical apparatus were not always diffusive. The condition is that it is important to know the properties of pharmacological substances with expected potentials of interaction, which are usually defined by pharmacologists for their practical applications in biosystem environment.

Presented procedure of analysis of medicine penetration is not a hospital recipe, but it is a formula showing difficulties which should lead to obtaining the hospital procedure.

This new proposal is also a challenge for pharmaceutical industry for undertaking the co-operation.

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SNORING AS A SIGN OF ABNORMALITY

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Abstract: Medical support is needed for subjects who snore to get a good night's sleep. This prospective study is aimed to show how sleep disorders influence a good night sleep. One of the common sleep disorder that affect people of all ages is snoring. Snoring sound could be a sign of cardiovascular disease. Correct interpretation remains a very significant problem in dealing with respiratory sounds, such as snoring and/or breathing. Acoustic characteristics of snoring sounds, which are approximately periodic waves with noise, can be analyzed by using a multidimensional voice program MDVP. Acoustic analysis techniques give information on the mechanism, loudness, intensity and sites of obstruction of upper airways. Further conclusions can be made by comparing simultaneously gathered acoustic and electrocardiographic signals.
Keywords: snoring, acoustic analysis, good night's sleep

Introduction

Snoring is a well-known sound generated by the vibrations of partially collapsed and unstable pharyngeal airway walls and the soft plate. Snoringis a breathing noise that appears during the inspiratory andsometimes also the expiratory phase of the respiratory cycle [2]. Moreover, snoring is looked at more as a symptom or sign of development of obstructive sleep apnea syndrome in the future. Snoring is a common sleep disorder that affects people of all ages, although it is most frequent for men and overweight people. This is related to body mass index (BMI), daytime sleepiness, and hypertension stroke or heart disease[3].

Among adults who snore, two kinds of snorers can be distinguished: occasional and habitual. These two kinds of snoring could have different influence on quality rest during sleep: usually an occasional snorer has good sleep and rest but a habitual snorer is often tired after all night sleep and does not have good quality rest.

Many things have influence on the acoustic phenomenon that is snoring. Sound of snoring might change during a single sleeping period and it may vary from night to night. Breathing by nasal, oral or both structures of upper airway, sleep stage, body position - these factors determinate quality of the sound. We must distinguish snoring from the other sleep sounds or noises. Objective analysis of snoring is very important for evaluating treatment.

Acoustic investigation

Snoring is produced in the vocal tract, similarly to speech. Thanks to that analogy, existing techniques for speech analysis have been applied to evaluate snoring sounds. Snoring sound was precisely and easily detected with use of a condenser microphone. Microphone was hung in front of the patient's mouth at a distance of about 15-20cm. The signal was recorded and sent through the analogue-digital converter directly to the computer system and subsequent analysis was performed.

Acoustic analysis of habitual and occasional snorers started with auditory analysis. For an individual who is an occasional snorer we can observe that his breath is very regular. In addition, only at the third hour of sleep regular sounds of snoring with very low intensity could be heard. On the other hand, a habitual snorer has an extremely diverse signal in the time domain which makes it difficult to interpret: throughout the entire night changes of snoring intensity could be heard and breathing is irregular.

There are numerous techniques and methods employed to measure tsnoring. Acoustic analysis techniques give information on the mechanism, loudness, intensity and sites of obstruction of the upper airways. Snoring has been analyzed in the frequency and time domain and it was defined with the set of quantitative sound parameters (table 1).

Table 1. Techniques and methods employed to measure snoring [7]

Spikes in sound intensity of breathing	
Maximum snoring intensity	
Mean snoring intensity	>45dB or >65dB
Number of snorers $\cdot h^{-1}$ of sleep	dB max
Number of snorers $\cdot min^{-1}$ of snoring	dB mean
Power spectrum	SI
Sonogram	SF
Formants structure	
LPC	

The transformation of data from the time domain to the frequency domain was carried out by the Short-Time Fourier transform algorithm implemented in a PC software called "snore", written in the Matlab programming environment. Sampling frequency of the analog-to-digital converter (44100 Hz) determines the maximum time duration of the sample. Frequency range of 12 kHz can completely describe the snoring phenomenon.Snoring sounds were analyzed using the short-time Fourier transform (STFT) to determine the frequency and content of local sections of the samples. It can be described using the following equation:

$$STFT_x^T = X(\tau,f) = \int_{-\infty}^{\infty} x(t)w(t-\tau)e^{-2\pi fi}dt \quad (1)$$

where $w(t)$ is the window function, commonly a Hamming window (width N = 353 samples), centered around zero, and $x(t)$ is the signal to be transformed. $X(\tau,f)$ is essentially the Fourier Transform of $x(t)w(t-\tau)$, a complex function representing the phase and magnitude of the signal over time and frequency.

Time variation of the frequency spectrum is realized by dividing the analyzed signal into short, overlapping segments (fig. 1). Signal in 10ms segments becomes stationary, so a short-time Fourier transform can be performed. After raising the resulting spectrum to the second power these segments can be combined. Time variation of the frequency spectrum is defined as square module of STFT [4, 5]. It can be described using the following equation:

$$G_x(t,f) = |\,\mathrm{STFT}_x = (t,f)|^2 \quad (2)$$

The STFT is a complete description of the signal and it is an important procedure for further analysis.

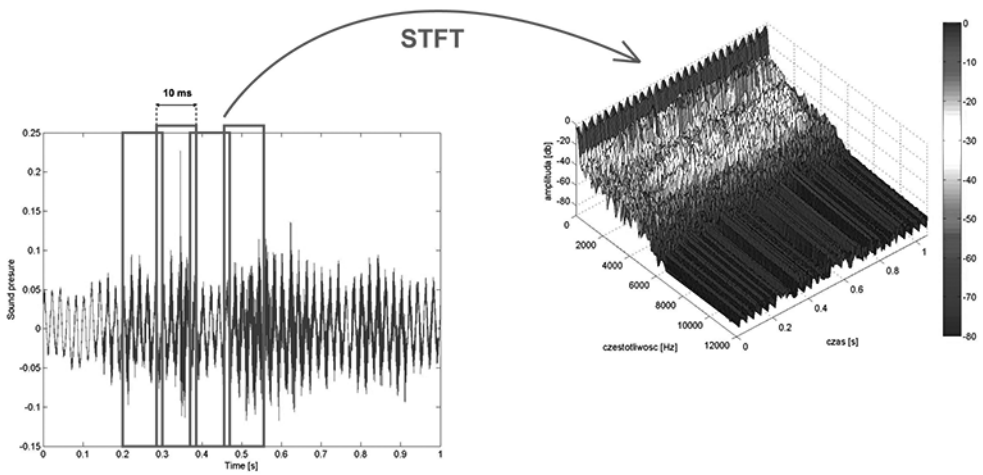

Fig. 1. Short time Fourier transform

Waveforms of snoring events over a period of 10s (fig. 2) were analyzed. Depending on the snorer there correspond to from two to four respiratory cycles. The subjects have very regular respiratory cycles, unlike typical snoring patients.

The frequency domain provides most important information from snoring sound, enabling power analysis and three-dimensional graphs [1]. The averaged spectrum shape of snoring event is represented with values (Hz) of its formants. Different conditions in which subjects and patients snore can affect formants range. Examining snoring sound signal during sleep, energy was mainly concentrated in low frequencies, below 6000Hz. The main components lie in the low frequency range, at about 130Hz. The spectrum shows a fundamental frequency and formants structure. Also the frequency spectrum changes in every snoring event or during respiratory cycle (fig. 3 and 4).

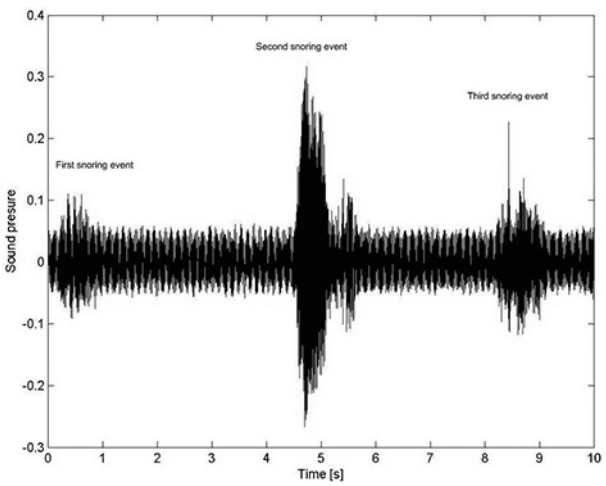

Fig. 2. Normalized waveform of three snoring events over a period of 10s

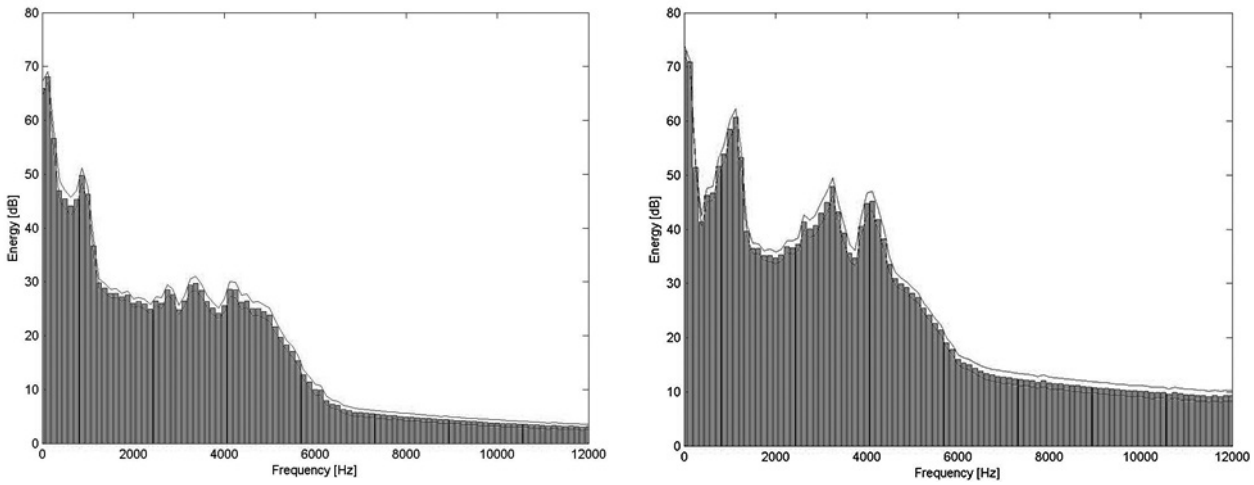

Fig. 3. The averaged spectrum shape of two snoring events
(left: second snoring event, right: third snoring event) for a single patient

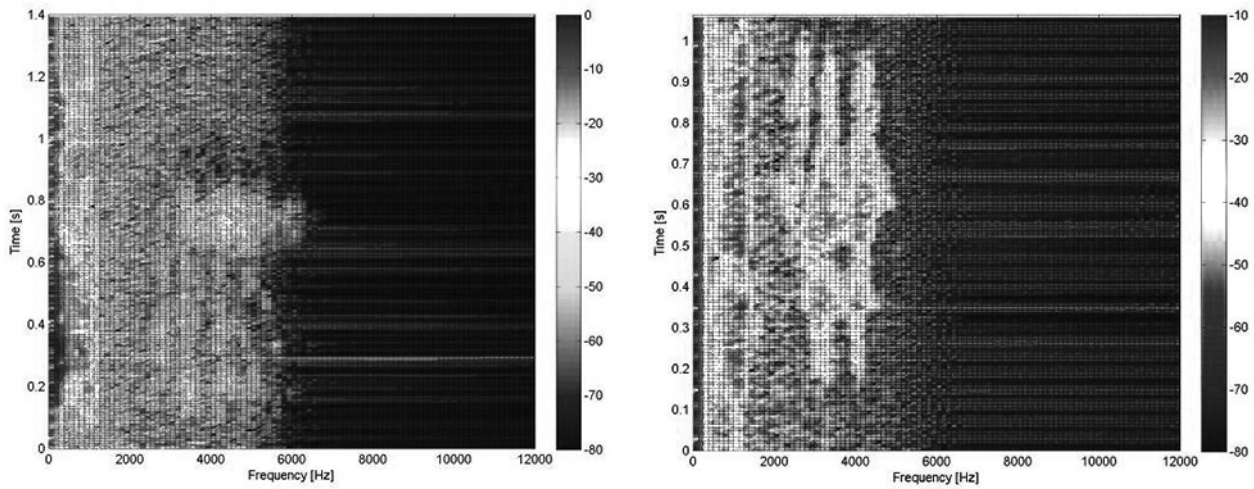

Fig. 4. Time variation of the frequency spectrum of two snoring events
(left: second snoring event, right: third snoring event) of a single patient

Frequency, bandwidth and amplitude levels characterize formants. The range of these parameters depends on the shape of the resonant cavities. According to Perez–Padilla et al[5], three types of snoring can be distinguished, namely oral, nasal and oronasal, which present different spectra. Acoustic analysis of vowels revealed decreased values of F3 and F4 formant levels and energy concentration in the lower range of frequencies, suggesting the presence of acoustic characteristics of nasal snoring. This is the most common resonance disorder.

Thanks to a complete acoustic analysis we are able to extract two- or three-dimensional acoustic images which represent time variability in each frequency band. This kind of presentation shows three types of information: in the time and frequency domain and amplitude sound level. In figure 5 normal and pathological signals are compared. It is noteworthy that the pathological signal has more transient components, especially at lower frequencies.

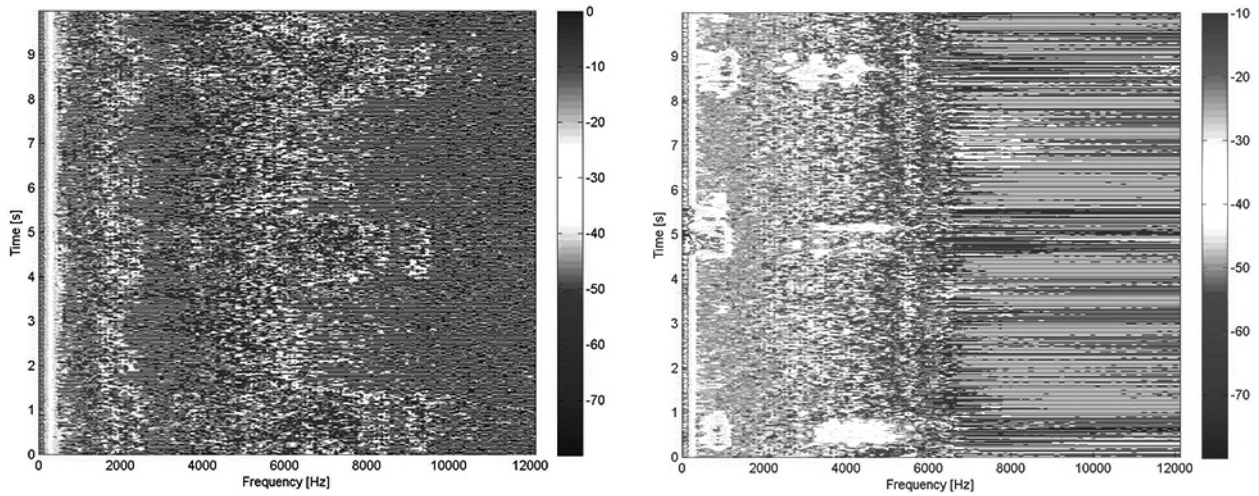

Fig. 5. left: STFT of a normal signal; right: STFT of an abnorm signal

The signal of a snorer presents weak formants, while the normal signal has more periodicity in low frequencies, and introduces stronger formants. Calculated parameters which form a vector of features of the abnormal sound were quantitatively compared with normal sound, which allowed drawing conclusions from the available data. The most important parameters were the fundamental frequency, moments M0-M2 and formants. The pathological importance of snoring has been related to its intensity (dB), maximal and mean intensity, number of breaths per minute of sleep, snoring frequency and formants structure. As it is presented in table 2 below, the mean energy is significantly higher for habitual snorers when compared to occasional snorers.

Table 2. Acoustic parameters of snoring sound

	Occasional snorer	Habitual snorer	Subject
Minimum Energy [dB]	44.92	47.20	45.56
Maximum Energy [dB]	61.02	58.67	47.88
Mean Energy [dB]	48.64	57.47	46.66
Standard Deviation [dB]	1.45	1.95	0.29
Median Energy [dB]	48.57	57.83	46.68
number of snorers/minute	7.00	10.00	0.00
number of breathing/minute	15.00	10.00	15.00
M0 [a.u.]	426.00	2620.00	427.00
M1[a.u.]	44.00	72.00	44.00
M2 [a.u.]	2939.35	6267.81	3149.44
F1 [a.u.]	127.00	125.00	127.00
F2 [a.u.]	2499.00	1249.00	1400.00
F3 [a.u.]	2748.00	1874.00	2374.00
F4 [a.u.]	3123.00	2998.00	2748.00

Acoustic characteristics of snoring sounds, which are approximately periodic waves with noise, can be analyzed also by using the multidimensional voice program MDVP.Multiple protocols of MDVP can show differences between markers used in an automatic analysis e.g. peak frequency, soft phonation index (SPI), noise to harmonics ratio (NHR), and fundamental frequency variation (vF0). Measurements (Meas.1-4) results compared with norms are shown on polar diagrams that are very easy to interpret.

Table 3. Parameters vF0, SPI, NHR for habitual snorer (male)

Parameters	Norm	Standard Deviation	Meas.1	Meas.2	Meas.3	Meas.4
vF0 [%]	0.94	0.43	68.73	45.26	43.26	43.32
SPI	6.77	3.78	7.49	6.37	0.63	5.09
NHR	0.12	0.01	3.55	1.54	2.79	3.79

Utilization of acoustic methods yields research results in a straightforward, graphical way. Proposed methods can support snoring individuals cope with this disorder. Based on this method, investigators can judge what kind of disorders they are dealing with. Acoustic diagnosis of sleep disorders, especially of the Obstructive Sleep Apnea Syndrome, is expected to provide a solution for snoring rehabilitation and making decisions concerning the future treatment and influence of various environmental factors on a good night sleep. Green color in the polar diagram shows the standard and red color represents the pathological changes in snoring event for a single patient(fig. 6). Parameters which describe snoring sound, presented in this easy and transparent way, can be clearly interpreted - the most important parameters of snoring sound like vF0 and NHR are above normal value.

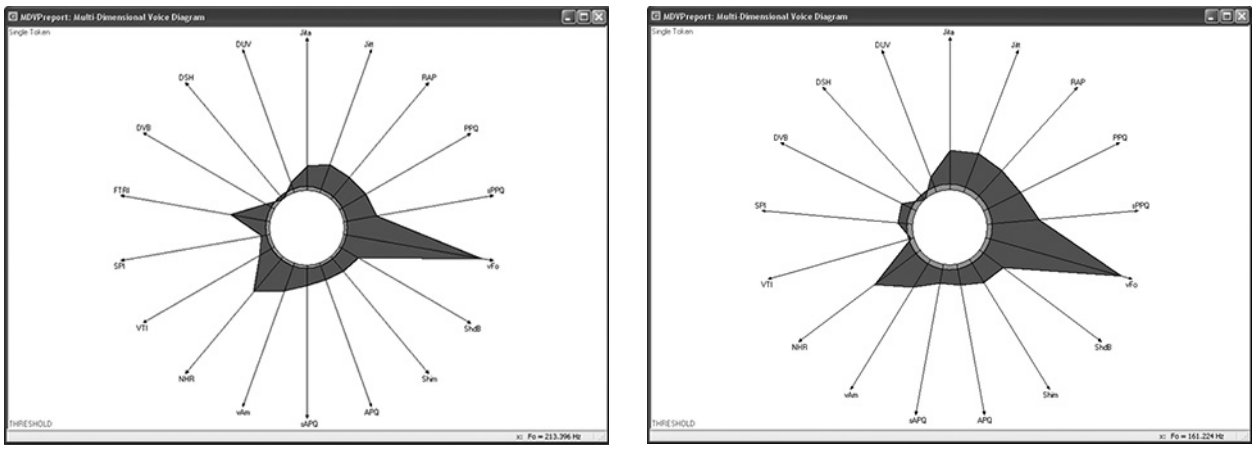

Fig. 6. Polar diagram of MDVP (left: second snoring event, right: third snoring event) for a single patient

Conclusion

Acoustic analysis techniques used in this work gave information about loudness and intensity of the snoring sound. Analysis of results led to the conclusion that the curved septum is a possible reason of obstruction of the upper airways.

Close examination of snoring sound signal during various stages of sleep demonstrated that light snorers snored evenly throughout all of them. The most interesting fact was that habitual snorers tend to snore more with maximum snoring intensity in the rapid eye movement (REM) sleep phase than in any other stage of sleep (every 90 minutes). Further conclusions can be made by comparing simultaneously gathered acoustic and electrocardiographic signals. The first study showed that during two thirty-minutes intervals, starting at 130 and 300 minutes after recording initiated, a significant decrease in Heart Rate Variability (HRV) can be seen, especially short term SDANN (Standard Deviation of the Averages of NN (Normal Sinus to Normal Sinus) intervals in all 5-minute segments of a 24-hour recording), which is in accordance with RMSSD (Root Mean Square Successive Difference - in heart period series is a time domain measure of heart period variability). During said intervals an increase of snoring sound intensity can also be seen. Snoring as a marker of abnormality can be only used in context of patient's medical history. The history and related diagnostic tests help to determine whether the patient has abnormalities or is just a healthy snorer without other disorders (habitual or occasional snorer). For snoring patients common abnormalities include sleep disorders, breathing disturbances or apneas during sleep and other daytime disturbances.

By combining results from the tests summarized in this paper with polysomnography tests and patient history files, physicians will be able to better diagnose the patient and indicate the functional nocturnal and daily disturbances. It is also crucial that as many characteristics of snoring events as possible are recorded, for instance were they recent or longstanding, continuous or intermittent, what was the sleeping arrangement, were there any movements of the body or legs during the sleep. In addition, factors like morning headaches, daytime sleepiness, alcohol consumption and smoking, as well as patient's lifestyle in general, should be monitored.

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LAPAROSCOPIC COLPOPEXY TECHNIQUE PERFORMED WITH LAPAROSCOPIC SUPRACERVICAL HYSTERECTOMY, TOTAL LAPAROSCOPIC HYSTERECTOMY, AND FOLLOWING TOTAL ABDOMINAL HYSTERECTOMY

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Abstract: Study objective: Our purpose was to assess a modification of laparoscopic colpopexy technique.
Design: Retrospective case analysis
Setting: University teaching hospital
Patients: A total of 28 patients with stage I, II and III organ prolapse.
Interventions: The rectovaginal space was dissected at the superior aspect of the posterior vaginal fascia and a mesh was sutured to this fascia
Measurements and Main Results: 28 patients completed a 36 months follow-up. No recurrences of the vagina vault prolapse was observed. Three cases of abdominal pain at the level of the trocars placing were recorded in a first week after surgery. Two of these were found in the group of LSH and one in the group of a hysterectomy history. There was no significant difference in blood loss, length of hospital stay and surgical or postoperative complications.
Conclusion: The modification of laparoscopic colpopexy, although technically challenging, may be learned quickly. It shows rapid improvement in operative time without subjecting the patient to undue risk. Further studies are required to determine the long-term tolerance and outcome of the procedure.
Keywords: colpopexy, laparoscopy, organ prolapse, vagina fault

Pelvic organ prolapse may occur in up to 50% of parous women. An estimated three out of four women with prolapse have a rectocele. This problem is associated with a 11% lifetime risk of requiring major surgery [1]. Laparoscopic approach and techniques have been applied to most abdominal route surgical procedures for treatment of urinary incontinence and pelvic organ prolapse. As was first reported by Nezhat et al., the application of laparoscopic abdominal sacral colopexy has increased [2]. The recurrence rate reaching 30% following traditional surgical repair of vaginal prolapse has consequently led to more frequent use of permanent mesh and grafts. The use of mesh for sacrocolpopexy for correcting apical vaginal prolapse has a reported success rate from 78 to 100% and median reoperation rate for prolapse of 4.4%. Nygaard et al. reported a mean rate of mesh erosion of 3.4% [3]. The study made by Culligan et al. reported a 27% mesh erosion rate when sacrocolpopexy was performed with hysterectomy, compared with 1.3% when concomitant hysterectomy was not performed [4].

Cundiff and Fenner reviewed and summarized outcomes after posterior colporrhaphy, site-specific repair, transanal repair, and rectocele repair with graft materials [5]. Anatomic cure ranged from 76% to 96% for posterior colporrhaphy and from 56% to 100% for site-specific defect repair. For example, a paravaginal defect repair (PVDR) involves repair of discrete defects in the anterior vaginal endopelvic fascia. The success of the abdominal paravaginal repair for correcting cystocele ranges between 76 and 97% [6].

Common indications for the graft implantation include: post partum patients, after previous failed surgery due to organ prolapse and for severe pelvic organ prolapse [7]. On the other hand, according to the International Urogynecological Association, contraindications such as host conditions may compromise the

vascular supply to the pelvic floor. These include history of pelvic radiation, poorly controlled diabetes, severe vaginal atrophy, systemic steroid use, and heavy tobacco use [8].

Materials and methods

The objective of this study was to assess the safety and feasibility of a laparoscopic approach for vaginal vault defect, using mesh placed along the round ligaments and sutured into the fascia of the rectus muscle.

We analyzed 28 patients diagnosed with symptomatic vaginal vault prolapse. All the women underwent a standardized pelvic examination to evaluate the stage of genital prolapse. This was done through the use of the Pelvic Organ Prolapse Quantification (POPQ), which is designed to assess the anterior vaginal wall prolapse (point Ba), uterine or vaginal vault prolapse (point C) and posterior vaginal wall prolapse (point Bp) on maximum Valsalva effort. One of the patients had urodynamic evaluation due to urinary incontinence [9].

All the patients in the study (except Group C) had history of vaginal bleeding due to uterine fibroids or menopausal hormone disorders. To rule out endometrial and cervical cancer among Groups A and B, endometrial biopsy and Pap smears were conducted respectively. There were no pathologic findings.

Among patients younger then 50 years of age without abnormalities on pelvic ultrasound, adnexae were left in place.

Preoperative bowel preparation was done in the evening before surgery. For prevention of deep venous thrombosis 4000U/daily s.q. of low weight heparinwas given, while cefuroxim (3g/daily i.v.) was used prophylactically against infection.

The patient was placed in a modified dorsolithotomy position with legs semiflexed and apart, and with arms by her side. A Foley catheter was introduced into the bladder and the patient underwent general anesthesia and endotracheal intubation.

The following technique was applicable to patients with mild to moderate vaginal vault prolapse. The patient was placed in a supine position and prepared and draped using a sterile technique. Laparoscopic approach for colpopexy can be performed with uterine extirpation or among patients who have a history of hysterectomy. Three incisions were made using trocars; one 10mm cut at the umbilical apex, another two 5mm cut bilaterally medial and finally just above the ileac crest.

We evaluated three groups of patients. Group A, which had colpopexy performed with LSH (laparoscopic supracervical hysterectomy), group B with TLH (total laparoscopic hysterectomy) and group C patients who have had a previous hysterectomy.

We used monofilament polypropylene mesh (Prolene, Ethicon, Johnson & Johnson, UK).

A larger 30cm x 30cm mesh was reduced to dimensions of 200 mm x 20mm, followed by a 150mm lengthwise cut at the centre as shown in Figure 1.

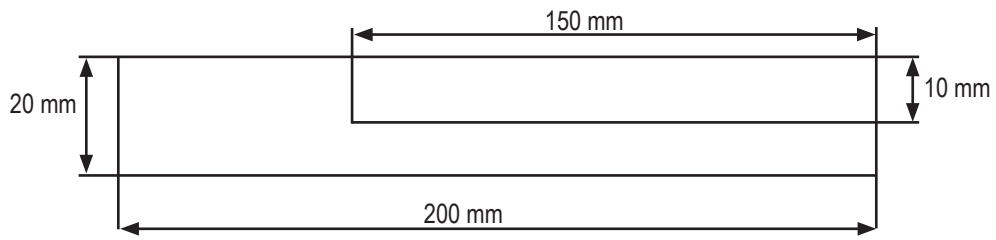

Fig. 1. Mesh was inserted into the peritoneal cavity using the 5 mm trocar.

1. Group A

The first stage in this group was the LSH procedure with or without bilateral adnexectomy. The uterine corpus was sectioned at the level of the isthmus above the uterosacral ligaments, using the monopolar-hook coagulator. In order to facilitate the placing of the mesh, the rectovaginal space was dilated between the uterosacral ligaments. Subsequently, the 50mm long part of the mesh was inserted into the tunnel and fixed to the vaginal fibromuscularis tissue with no. 0 Vicryl, using the laparascopic technique. On the other hand, in the case of the rectocoele patient mesh was attached to the vaginal fibromuscularis laprascopically, in addition, to the vaginal approach.

The mesh was fixed superiorly to the cervical stump and onto each of the uterosacral ligaments, similar to the McCall procedure. The branches of the mesh were then lead out thru the trocar's tunnels, below the fascia of the rectus and oblique muscles and along each of the round ligaments. The branches of the mesh were covered with peritoneum and part of the round ligament. Then they were attached to the muscle fascia using 1 or 2 stitches of no. 1 Ticron surgical threat.

2. Group B

The main procedure in this group was the TLH, with or without adnexectomy. The first stages of TLH were similar to those in LSH. At the level of the uterine vessels, the parametria was coagulated and removed. The uterus was then amputated from the vaginal fornices using a monopolar-hook coagulator. The incision of the vaginal fornices was performed along the visible margin of the sealant cap. Subsequently, the uterus was pulled out vaginally and the vagina was closed with continuous sutures. Consequently, the uterosacral ligaments were located and the area between them was dissected. The mesh was then attached to the vaginal stump. The next stages were similar to those described for Group A.

In the case of the rectocole, mesh was attached to the vaginal fibromuscularis using the vaginal approach with the aid of the laparascopic technique, as was done in Group A.

3. Group C

Patients in this group have had a previous hysterectomy. Mesh placing was performed similar to that as in Group B. In the case of the rectocele, we attached the mesh to the vaginal fibromuscularis membrane using the vaginal approach and, as in Group B, it was done laparoscopically.

4. Postoperative

Heparin was continued until complete patient mobilization and the bladder catheter was maintained for 24 h postoperatively. Patients were discharged after 3 days in Group A and C and after 2 days in Group B.

We evaluated the primary outcome with the POP-Q system. The secondary outcome was evaluated for recurrent vaginal vault prolapse to the introitus or beyond it and for symptom resolution. Each patient was seen 1 month, 3 months and then annually after the procedure.

Changes in symptoms were classified as resolved, persistent, or new onset from baseline to the last follow-up visit.

Results

From February 2005 to January 2009 we treated 28 patients. Demographics and clinical characteristics are presented in Table 1. Mean age and standard deviation (SD) at enrollment was 49,93 (4,19) years, with a range of 42-58 years. Ten of the patients presented a history of hysterectomy, but none with previous pelvic floor surgery. In Group A six patients delivered twice, five once and one for three times. In Group B five patients delivered twice and one once and in Group C six patients delivered twice, two once and two for three times. We performed vaginal vault reconstruction with LSH in 12 patients, with TLH in six patients and in ten patients with a history of hysterectomy. In two of the cases, in Group A, additionally we performed TVT for stress incontinence, which was diagnosed through urodynamic examination. The vaginal prolapse was completely treated in all patients and no recurrences were observed after a follow-up of 36 months. We recorded six cases (three in Group A, one in Group B and two in Group C) with abdominal pain, presenting one week after surgery, at the level of the placement of the trocars (Table 3). It was successfully resolved with 50mg of Ketonal repeated twice a day for one week. In one of the cases, in Group B, urinary retention in the amount of 200ml was noticed. This was resolved by the introduction of a Foley catheter for 24 hours. We encountered nor bladder nor rectal perforation, blood transfusions, infections, pelvic hematomas, deep vein thrombosis or pulmonary embolisms.

Three patients in Group A and two in Group C were premenopausal. Six in nine patients in Group A received hormonal replacement therapy, compared to 2 and one patient in Groups B and C.

Table 1. Demographics, previous surgeries

	Group A n= 12	**Group B n= 6**	**Group C n= 10**
Age (mean)	46,83	52,83	51,90
Menopausal status			
Premenopausal	3	0	2
Postmenopausal with HRT	6	2	1
Postmenopausal without HRT	3	4	7
Prior hysterectomy or urogyne-cologic or pelvic surgery	0	0	10
Parity			
1	5	1	2
2	6	5	6
3	1	0	2
Preoperative prolapse POPQ			
Stage I	0	0	4
Stage II	9	4	6
Stage III	3	2	0
Urinary incontinence			
Stress	2	0	0

Descriptive statistics of measurable variables are shown in Table 2 and 3. Mean patient's age was 49,93 years old, mean operation time 55,18 minutes (min) and mean estimated blood loss 118,04 milliliters (mL).

Table 2. Descriptive statistics of measurable variables (age, mean operation time and estimated blood loss)

	Mean	SD	Minimum	Maximum	N
Age	49,93	4,19	42,00	58,00	28,00
A (for LSH)	46.83	2.75	42,00	51,00	12,00
B (for TLH)	52,83	1,83	51,00	55,00	6
C (for after_hyster)	51,90	4,33	45,00	58,00	10
mean operation time (min)	55,18	7,63	48,00	70,00	28,00
A (for LSH)	51,50	2.96	48,00	57,00	12,00
B (for TLH)	68,83	1,32	67,00	70,00	6
C (for after_hyster)	51,40	2,17	49,00	55,00	10
mean estimated blood loss (mL)	118,04	22,66	85,00	160,00	28,00
A (for LSH)	120,00	12,06	100,00	140,00	12,00
B (for TLH)	144,16	15,62	120,00	160,00	6
C (for after_hyster)	100,00	20,13	85,00	159,00	10
mean length of hospital stay	3,10	0,46	2,45	3,29	28
A (for LSH)	2.16	0,38	2,00	3,00	12
B (for TLH)	3.33	0,51	3,00	4,00	6
C (for after_hyster)	3.20	0,42	3,00	4,00	10

Table 3. Descriptive statistics of variables eg. “Operation type”, “POPQ”, “Diagnosis”, “pain at the level of fascia after 1 week”, “Parity”

	Value	Frequency	Percent of Total
Operation type*	A (LSH)	12	42,86
	B (TLH)	6	21,43
	C (after_hyster)	10	35,71
POPQ**	3	5	17,86
	2	19	67,86
	1	4	14,29
Diagnosis***	0	10	35,71
	1	9	32,14
	2	9	32,14
pain at the level of fascia after 1 week****	0	22	78,57
	1	6	21,43
Parity (number of deliveries)*****	1	8	28,57
	2	17	60,71
	3	3	10,71

* Operation type: LSH – laparoscopic subtotal hysterectomy; TLH – total laparoscopic hysterectomy; after hysterectomy
** POPQ (pelvic organ prolaps quantification): 1 stage; 2 stage; 3 stage
*** Diagnosis: 0 – prolapsus; 1 – Mayoma; 2 – abnormal bleeding
**** Pain at the level of fascia after 1 week: 1 – pain; 0 – without pain
***** Parity: 1 – once, 2 – twice; 3 – for three times delivered

The Pearson Chi-Square Test was used for assessing dependences in contingency tables between particular immeasurable variables (Table 4).

Table 4. P-values calculated on the basis of the Pearson Chi-Square Test

	Partity	Pain at the level of facia after 1 month	Diagnosis	POPQ
Operation type	0.475	0.912	0.001	0.035
Partity	-	0.124	0.687	0.065
Pain at the level of fascia after 1 week	-	-	0.999	0.505
Diagnosis	-	-	-	0.085

Details for variables with statistical significance:

Table 4a. Operation type – Diagnosis

Operation type	diagnosis			Total
	0	1	2	%
LSH	0 0.00	8 28.57	4 14.29	12 42.86
TLH	0 0.00	1 3.57	5 17.86	6 21.43
after_hyster	10 35.71	0 0.00	0 0.00	10 35.71
Total	10 35.71	9 32.14	9 32.14	28 100.00

There were found statistical significant dependences between "Operation type" and "Diagnosis" (p=.001, α=.05) and "Operation type" and POPQ (p=.035, α=.05). Diagnosis of prolapsus indicated LSH procedure in 28,57% cases, vs abnormal vaginal bleeding for TLH in 17,86% (Table 4a).

Table 4b. Operation type – POPQ

Operation type	POPQ			Total
	1	2	3	%
LSH	0 0.00	9 32.14	3 10.71	12 42.86
TLH	0 0.00	4 14.29	2 7.14	6 21.43
after_hyster	4 14.29	6 21.43	0 0.00	10 35.71
Total %	4 14.29	19 67.86	5 17.86	28 100.00

POPQ 2 indicated LSH procedure in 32,14%, vs 14,29% for TLH and 21,43% after hysterectomy (Table 4b).

Table 5. P-values calculated on the basis of the Kruskal-Walis Test

	Age*	Mean operation time*	Mean length of hospital stay*	Mean estimated blood loss*
POPQ	0.34	0.56	0.67	0.13
Pain at the level of fascia after1 week	0.48	0.34	1	0.29
Diagnosis	0.0028	0.0111	0.0047	0.046
Parity	0.83	0.58	0.39	0.32
Operation type	0.0017	0.0008	0.001	0.0007

* variable does not follow a specified theoretical – normal distribution

Discussion

Statistical significance among mean values of quantitative variables as: "age" (p=.0017, α=.05), and qualitative – "Operation type" variable is observed (Table 5). It suggests that the age and the operation type are correlated and mean age for LSH was 46,83, vs TLH 52,83, vs after hysterectomy 51,90.

Mean operation time (p=.0008, α=.05) depended from operation type (mean time for all operations was 55,18 min vs 51,50 for LSH vs 68,83 min for TLH and 51,40 for after hysterectomy). We found statistically significant correlation between operation type and mean length of hospital stay (p=.001, α=.05), for LSH it was 2,16 days vs 3,33 for TLH and for 3,20 after hysterectomy. Mean estimated blood loss (p=.0007, α=.05) and operation type were statistically significant, with mean value for LSH 120 mL vs 144,16 mL for TLH and 100 mL for after hysterectomy (Table 5).

As with all of the other apical support procedures, it is difficult to quantify the success rates because of wide variation in outcome measures and definitions used for success.

Some studies focus on support of the vaginal apex, while others consider overall vaginal support. This is more complicated for abdominal repairs, in which posterior vaginal wall support procedures are often deferred, and there is a variation in concomitant procedures for anterior wall support and urinary incontinence.

Most of the studies use synthetic mesh grafts, which offer durable repairs. Several characteristics of synthetic meshes appear to affect the prevalence of erosion, including pore size, filament type, and weave. The monofilament, macroporous soft meshes (polypropylene) seem to be the best for use in colpopexy. A recent randomized control trial provides level I evidence that allogenic fascia lata is inferior to synthetic mesh (68% versus 91% respectively at 1 year) for sacral colpopexy [10].

Sacral colpoperineopexy is a modification of sacral colpopexy aimed at correcting a combination of conditions, including apical prolapsed, rectocele, and perineal descent. During ascral colpoperineopexy, a continuous graft is placed from the anterior longitudinal ligament to the perineal body. This can be accomplished either through a total abdominal approach or a combined abdominal and vaginal procedure. Cundiff et al. displayed good anatomical support of the vaginal apex, posterior wall, and perineum over short-term follow-up for 19 women undergoing sacral colpoperineopexy [11]. Defecatory dysfunction symptoms completely resolved in 66% of patients. Sullivan et al reported outcomes for a slightly different variation of sacral colpoperineopexy involving attachment of Marlex mesh to the perineal body using a needle carrier [12]. The failure rate was 25% and the mesh erosion rate was 5% for 205 patients with up to 10-year follow-up.

Colombo and Milani performed a retrospective case-control study using 62 cases of sacrospinous ligament fixation and 62 matched controls undergoing modified McCall culdoplasty [13]. There was no statistically significant differences in postoperative objective support, even for women with procidentia. The authors concluded that McCall culdoplasty was equally efficacious as sacrospinous ligament suspension with less morbidity, and sacrospinous suspension should no longer be considered as a treatment in patients with uterovaginal prolapsed.

Laparoscopic sacral colpopexy is an attempt to make sacral colpopexy less invasive with a quicker recovery, similar to vaginal surgery. Complication rates, operative time, duration of hospitalization, recovery time, postoperative recovery, and costs have not been prospectively compared for laparoscopic and open sacral colpopexy.

There is no available literature for laparoscopic colpopexy modification described in the article. This technique, as a whole, allows a complete anatomical repositioning of the pelvic organs that influence their various functions and prevent any possible new prolapsed.

Laparoscopy is a viable approach for the surgical treatment of urogenital prolapse. It is a minimally invasive surgery that requires a short hospital stay. Few complications were observed. Laparoscopic colpopexy with the addition of mesh is particularly indicated for women with vaginal vault prolapse who would like to improve their quality of life. This unique approach can be used concomitantly with LSH or TLH. It is also helpful for patients with a history of hysterectomy, for whom laparoscopy will allow minimal postoperative pain and shorter hospital stay.

The obvious weaknesses of our report include the small patient number and the short-term follow-up.

Conclusion

The large number of corrective surgical techniques described in the literature for genitor-urinary prolapsed only proves that there is still no consensus on this issue.

We believe that the positive results of this minimally invasive procedure could be ascribed to the use of the typical laparoscopy technique. It must, however, be performed by an experienced surgeon.

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EFFECTIVENESS ANALYSIS OF SELECTED ATTENTION MODELS

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Abstract: The aim of this study was an empirical verification of five different computer models of exogenous human attention. These models allow to predict the likely fixations on a static image and calculate saliency maps which show how distinctive the image elements are.
The basis for the verification of the models was a modified cuing task, originally developed by Posner. Using the algorithms provided by the authors of the models an analysis of selected images was performed. Then the effectiveness of the models was verified in experimental studies: an image was presented to the subject, then on this image a visual stimulus appeared and the reaction time was measured. It was assumed that the emergence of a stimulus at the point where previously an attention attracting element appeared would accelerate the reaction.
The study was conducted on a set of over 300 different, emotionally neutral, natural images. Obtained results indicate the potential usefulness of the proposed schema for testing the efficiency and refinement of attention models.
Keywords: attention modeling, saliency, the Posner's paradigm

Introduction

One of the most astonishing human capacities is the ability to analyze the visual field in real time. Intermediate and higher visual processing allows to select the most relevant information from all available visual stimuli which are then further processed. Searching can be guided by the distinctive physical features of image elements (bottom-up model of attention), as well as can base on subject's knowledge and depend on the task and desired item (top-down model of attention [1]). The models of top-down and bottom-up attention are two essential components in a contemporary frame-work for attention deployment [2].

Some researchers claim that the top-down attention, based on variable selection criteria, is probably more powerful than the bottom-up attention [2]. It is dependent on the task that is performed and on sought elements in the visual field. What is more, it can be volitionally controlled and much of attention is guided by higher brain areas [2][11]. However, volitional control is connected with a timing cost and it takes 200 ms or more to move the eyes to the interesting element [2].

In contrast, the bottom-up attention is a response to a sheer, severe sensory stimulation [11]. Some elements in the visual field stand out from the background automatically, regardless of a subject and an experience or a task [2]. The saliency of an element depends on the background and the context [12]. Particular item attracts attention on the basis of a "pop-out" mechanism: it somehow pops up, stands out from the background. Probably this is computed in terms of centre-surround mechanisms, which are a neuronal response to image differences between a center and a broader surround. Moving eyes for a particular, salient element is fully automatic and takes about 20-50 ms [2].

In recent years, studies and researches of mathematical, computational models of visual attention are developing [5]. Most researchers focus on the analysis and simulation of bottom-up attention processes. Much less work was done in the field of top-down attention [2]. Models of bottom-up attention, consisting on an analysis of the image characteristics without taking into account the knowledge of the observer, are easier to construct. Numerous, detailed mathematical models that allow to compute so-called saliency maps, depicting image elements standing out between others, thus in the assumption attention-grabbing, have been already established. The effectiveness of the models is usually verified in terms of free viewing, by comparing the human fixations, obtained from the eye-tracking device, with the results of model calculations. It is assumed that the movement of the eyes follows attention. Some of the models concentrate on simulating brain processes, when the main aim of the others is to obtain results similar to those recorded from subjects [3].

Such models have many potential applications. They can be used in artificial vision systems, robotics, navigation systems as well as in automatic detection of desired objects on the image or in space [2]. In addition, they may help in the detection of certain diseases and human disorders.

In this study a specific approach to attention models evaluation was proposed and tested. It relies on the Posner's cuing task [4]. It allows testing attention models without the eyes movement registration. In the original Posner's task examined subjects had to focus eyes on the fixation point and to react without eyes movements as quickly as possible when the light stimulus appeared. Some test ran with a clue, which pointed to the expected locus of the stimulus. If the pointer showed the correct location, the response times was shorter. If the pointer gave the wrong information, then the longer reaction time was observed. In this study the intensity of saliency was treated as a clue. Thus the basis for the evaluation of models was an assumption that more accurate models allowed a better prediction of reaction time in regions of different levels of saliency.

Models of attention

It this study 5 saliency models were analyzed and verified: from simple, based on image color statistics, to more complex ones, which are based on various image characteristics and simulate learning mechanisms. The following chapter contains a short description of each model putting the main emphasis on the manner of action.

Zhai & Shah [7]

The model allows to determine saliency maps using color statistics of images. The algorithm has linear computational complexity with respect to the number of image pixels and therefore is well suited for real time image processing. The saliency map of an image is build upon the color contrast between image pixels. The saliency value for a single color component (cc) for an image pixel I_k, is defined as:

$$S_{cc}(I_k) = \sum_{i=0}^{255} f_i^{cc} \cdot D(i, I_k) \quad (1)$$

where: $f^{cc}(i)$ is the histogram for a given color component (red, green or blue) and $D(i,I_k)$ is a color distance map. The saliency value for the image pixel I_k is calculated as a sum of saliency values for red, green and blue color components:

$$S(I_k) = S_R(I_k) + S_G(I_k) + S_B(I_k) \quad (2)$$

The saliency value is higher for pixels which color is rare. A more detailed description of this model and of its application (a spatio-temporal model of attention) is available in [7].

SUN ICA & SUN DOG [3]

The SUN (Saliency Using Natural statistics) model is based on a Bayesian probabilistic framework. Zhang et al. assume that the visual system must actively estimate the probability of a target at every location given the visual features observed. Let z denote a point in the visual fieed (in this application z corresponds to a single image pixel), let the binary random variable C denote whether or not a point belongs to a target class, let the random variable L denote the location (the pixel coordinates) and let the random variable F denote the visual features of a point. Saliency of a point z is then defined as:

$$s_z = p(C=1 | F=f_z, L=l_z) \quad (3)$$

where f_z represents the feature values obsved at z and l_z represents the location of z. This probability can be calculated using Bayes' rule:

$$s_z = \frac{p(F=f_z, L=l_z \mid C=1)p(C=1)}{p(F=f_z, L=l_z)} \quad (4)$$

When some assumptions are made (all details in [3]) equation (4) reduces to:

$$\log s_z = -\log p(F=f_z) \quad (5)$$

which is the definition of bottom-up saliency used in the SUN model. There are two key factors that affect the final result of a saliency model when operating on an image: the feature space and the probability distribution of the features. In SUN the features are calculated as responses of biologically plausible DoG (Difference of Gaussians) linear filters and responses to filters learned from natural images using independent component analysis (ICA).

The probability distribution is calculated in two steps. First, for a series of natural images the filter responses (features) are calculated and an estimate of the probability distribution is obtained. Then the distribution is parameterized by a zero-mean generalized Gaussian distribution. This can be viewed as a learning mechanism and use of prior knowledge.

Computing the saliency map for a given image consists of two steps. First, the filter responses are calculated. Then this values are compared with the estimation of the probability distribution; the difference is the measure of saliency. The greater the difference, the higher the saliency value for the image pixel.

Bruce & Tsotos [8]

The model is based on the premise that the highest saliency values are assigned to the most informative regions of the image. Therefore, it is analyzed how the content of the given image region is unexpected regarding to its surroundings. The conspicuity map is calculated using the Shannon's Self-Information: $-log(p(x))$, where $p(x)$ is the overall likelihood. In this method, for the whole image, responses to several ICA filters are determined and then an estimate of the probability distribution is created. As the most salient are recognized these image regions where the joint likelihood of filter responses is low. Details of this method are described in [8]. It is worth to notice that this method is similar to the one described in Chapter 2.2. In both models, as the most salient are recognized regions in which the response to filters

differs much from the prior calculated probability distribution. In the SUN method the distribution is calculated on the basis of a training set of images and in the Bruce & Tsotos method on the basis of the analyzed image. Additionally, authors of the method noticed that the proposed computational scheme results in an architecture with cells and connectivity reminiscent of that appearing in the primate visual cortex.

Itti, Koch & Niebur [1]

The saliency model proposed by L. Itti, C. Koch and E. Niebur from California Institute of Technology in 1998 is one of the most frequently quoted and evaluated models for calculating conspicuity maps of images. The basis of the method is an assumption that attention is directed in different parts of the visual field according to the image parameters (intensity, color, orientation, texture). The input image is analyzed using three features maps: intensity, color and orientation. Each map is then scaled to nine spatial resolutions using dyadic Gaussian pyramids (from scale 1:1 to 1:256). The features are computed by a set of linear center-surround operators akin to visual receptive fields. Typical visual neurons are most sensitive in a small region (center), while stimuli presented in a broader, weaker antagonistic region (surround), concentric with the center, inhibit the neuron's response. Center-surround is implemented in the model as the difference between fine and coarse scales. The center is a pixel at scale $c \in \{2,3,4\}$, and the surround is the corresponding pixel at scale $s = c+\delta$, $\delta \in \{3,4\}$. The across-scale difference between two maps is obtained by interpolation to the finer scale and point-by-point subtraction.

Given the input image in RGB color space the following features are considered: intensity ($I = (r+g+b)/3$) and colors: red ($R = r -(g+b)/2$), green ($G = g -(r+b)/2$), blue ($B = b -(r+g)/2$) and yellow ($Y = (r+g)/2 – |r-g|/2 – b$). The first set of feature maps is connected with intensity contrast, the second with color channels (so-called "color double-opponent" system) and the third with orientation (filtering the intensity map with Gabor filters of different orientations). In total, 42 feature maps are computed: 6 for intensity, 12 for color and 24 for orientation.

The saliency maps of each feature are then combined using a special normalization operator. It globally promotes maps in which a small number of strong peaks of saliency is present while globally suppresses maps which contain numerous comparable peak responses. Finally, the algorithm returns three conspicuity maps (intensity, color, orientation) and a global saliency map. All details of the algorithm are described in [1]. Additionally, Itti et al. proposed a method for predicting successive gaze fixations using local maxima of the saliency map and a 2D layer of leaky integrate and fire neurons.

Methods

Implementation of the models

For all five models the conspicuity maps were created. For the models SUN ICA, Bruce & Tsotos and Itti, Koch & Niebur functions and scripts in Matlab environment, provided by the algorithms' authors, were used to create the conspicuity maps. It is worth to mention that the Saliency Toolbox [6] of the Itti, Koch & Niebur method [1] presents the finest functionality and is being constantly developed. The rest of models – SUN DOG and Zhai & Shah – were implemented in Matlab on the basis of authors' publications [3][7]. Sample saliency maps for each model are presented in Figure 1.

Fig. 1. Sample saliency maps for five attention models: a) original image, b) Zhai & Shah, c) SUN ICA, d) SUN DOG, d) Bruce & Tsotos, e) Itti, Koch & Niebur

Models' verification

Subjects:

20 persons (10 women, 10 men) took part in the experiment. The subjects age was in range 16 to 50 years.

Experiment

In the study a modification of the cuing task developed by Posner [4] was proposed to verify the attention models. An image, which acted as a cue, was presented to the subject on laptop screen. There was no precise task as to create condition of free viewing. After a defined time (SOA – stimulus onset asynchrony) in one of nine possible locations in the image, the stimulus appeared. The stimulus was a red dot on a black background. The possible locations of dots in the image are shown in Figure 2. When the subject noticed the dot, he should have reacted immediately by pressing a button on the computer keyboard. A shorter reaction time was assumed when the stimulus appeared in a location close to the actual focus of attention. Similarly, a longer reaction time was assumed in the situation when a stimuli appeared in the area where attention was not focused currently.

Fig. 2. Possible locations of the stimulus

The proposed research methodology required making several assumptions. The image was divided into nine, equal rectangle areas; the stimulus could appear in the center of any of the nine regions. The SOA times were set as 75ms, 200ms and 600ms. If the dot appeared in the place where the subject's attention was focused, the response time should have been shortened for the SOA = 75 ms and 200 ms and lengthened for SOA = 600 ms. The assumption of a longer reaction time in last condition was connected with the phenomenon of inhibition of return. The interval between consecutive images was varied (1000 ms, 1500 ms or 2000ms) to prevent a development of automatic reaction to stimuli. Between the images, a white cross in the center of a black background was displayed as a point of concentration at which the subjects had to look between images' exposures. A scheme of the experiment is presented in Figure 3.

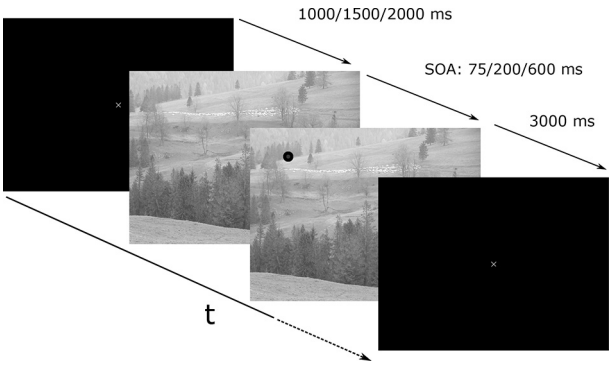

Fig. 3. Scheme of the preformed experiment

For each subject a separate experiment was randomly generated. It consisted of 50 training and 250 test photographs of natural, nonemotional scenes taken by authors. The images were not framed and showed no particular objects. This was done because of the suggestion presented in [3] that framed photographs usually have the highest saliency concentrated in the center.

The aim of the training session was to enable subjects to become acquainted with the methodology of the experiment. The results obtained in these trials were not taken into account in the final analysis. The proper session was divided into two parts of 125 images each, with an interval between them, what allowed the subject to rest. The sequence of images was random for each person, as well as the place of the emergence of the stimulus. SOA and intervals between successive images were random as well. For experiment generation and results analysis, scripts developed in the Matlab environment were used, and to perform the experiment dMdX program [9] developed at the University of Arizona was employed. For each of the 20 subjects 300 measurements of reaction time were obtained (50 trial and 250 test images).

Saliency measure calculation

The experimental data were analyzed by comparing the reaction times of the subject and the saliency measure for a region where the cue appeared. Because of the proposed methodology (Chapter 3.2) several methods for calculating the saliency measure in each of the nine image regions were designed and implemented. The methods were divided into two groups: based on the simulated gaze fixations mechanism and based directly on the saliency map. In the first case a simplified version of the gaze fixations prediction method proposed in [1] was used. The method consisted of two steps: finding the global maximum on the saliency map, recognizing it as a fixation point and then setting the conspicuity values in the surround of this point to 0, which simulated the inhibition of return mechanism. In the described manner the first 5 and 30 fixations locations were determined. Subsequently, to each of the 9 image regions the saliency measure was assigned. In the first method (method 1) the maximal value was set for the region with the first fixation and for other

regions the saliency measure was set to 0. The second method (method 2) was based on generating 30 successive fixations and calculating the saliency measure for each image region as an weighted sum. The first fixation was assigned a weight of 30^2 and the following of 29^2, 28^2 etc.

Additionally two methods of calculating the saliency measure, based directly on the saliency maps, were proposed. The first method (method 3) used as the measure mean saliency in a region. It was a simple approach, but its main drawback was that it favored regions with large areas of medium level of saliency. That is not consistent with the theory of attention attracting mechanism, in which small areas with high conspicuity are favored. The second measure (method 4) was based on calculating mean saliency in the surround (a circle of radius 26 pixels) of the global maximum for each region.

In the first step of the performed data analysis, saliency maps for all 300 were computed using each of the five considered models. Maps generated by the Zhai & Shah, SUN ICA, SUN DOG and Bruce & Tsotos methods were processed using the morphological top-hat operation [10] with a 25x25 square structuring element, which allowed to emphasize local maxima. For the last method: Itti, Koch, Niebur, which generates maps significantly different from others (Fig. 1), this procedure was not justified. Then the maps were normalized to range (0;1) and the described saliency measures were calculated.

Results and discussion

Results for the method 1

For all models and different SOA the mean reaction time was calculated for the consistent and inconsistent condition separately. The consistent condition was the case when the stimulus appeared in the region where the saliency value was equal to 1. The inconsistent condition was when saliency value was equal to 0. The analysis of variance (repeated measure ANOVA) was used to determine the impact of the SOA and condition (independent variables) on the reaction time (dependent variable). Results for all models are presented in Table 1 and in Figure 4. For all the models, a null hypothesis that there was no relationship between the reaction time and SOA is false ($p<0,05$). For none of the models the null hypothesis, that there was no relationship between the reaction time and the saliency in a given region, cannot be rejected. The longest reaction time was observed for the shortest SOA. The results that for SOA = 75 and 200 ms the reaction time for the inconsistent condition is shorter then for the consistent one was close to the level of significance but did not reach it. For SOA = 600 ms the reaction time for the consistent condition is shorter then for the inconsistent one. This was an opposite result to the expected one.

Table 1. Results of ANOVA for all examined models

Model		F	DF		p
Zhai & Shah	SOA	14,06755	2	17	0,0002486
	Condition	0,45984	1	18	0,5063235
	SOA * condition	0,385227	2	17	0,6860858
SUN ICA	SOA	43,04244	2	17	0,0000002
	condition	0,047254	1	18	0,8303569
	SOA * condition	2,78097	2	17	0,0901808
SUN DoG	SOA	17,92765	2	17	0,0000649
	condition	1,054968	1	18	0,3179714
	SOA * condition	0,207856	2	17	0,8143580
Bruce & Tsotos	SOA	13,93739	2	17	0,0002611
	condition	0,497765	1	18	0,4895147
	SOA * condition	1,032445	2	17	0,3774196
Itti, Koch & Niebur	SOA	18,82289	2	17	0,0000489
	condition	0,382908	1	18	0,5438033
	SOA * condition	0,112775	2	17	0,8940142

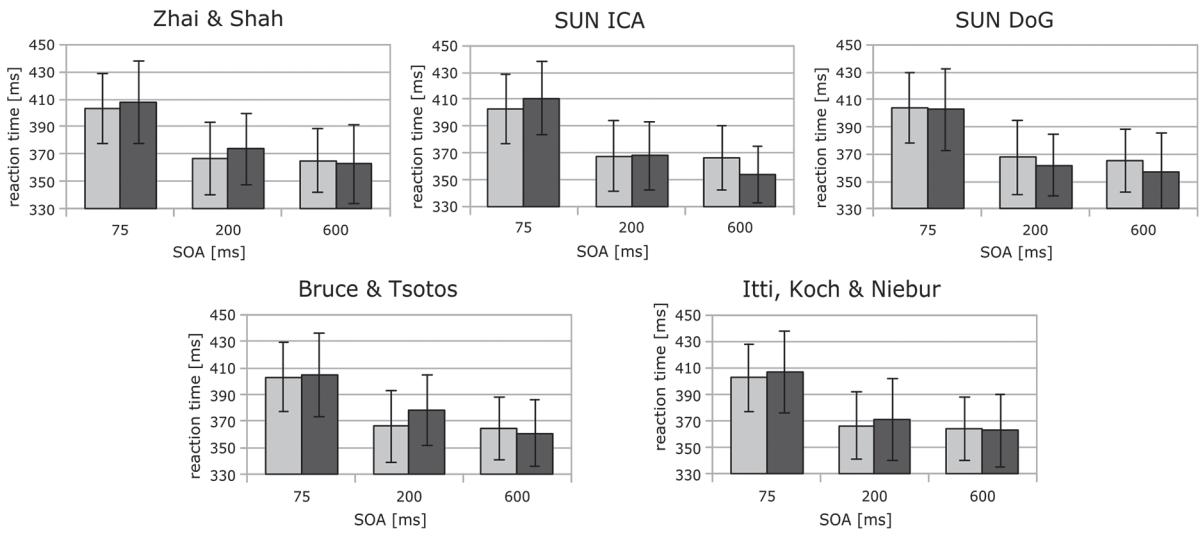

Fig. 4. Mean reaction time for different condition (light gray – inconsistent, dark gray – consistent) and SOA for all examined models

Additionally, for this method the mean reaction time for both conditions for each subject separately were calculated. The results were very noisy, strongly depended on the subject and test conditions. For each person, the difference between the reaction times for both conditions was very small and insignificant.

Results for methods 2,3,4

The linear regression analysis was performed for each method. The independent variables were SOA and saliency value. The dependent variable was the reaction time. The obtained results indicate that there is a dependency between SOA and the reaction time: the longer SOA is, the shorter reaction time is measured (the effect is statistically significant in all cases). This is a opposite result to the expected one. It could be connected with the time required for the reaction initialization. This effect should be examined in the future. Results are presented in Table 2.

Table 2. Sample results of linear regression for all models and methods

Model (method)		β	Std. Error	t value	Pr(>|t|)
Zhai & Shah (2)	Constant	402.515117	3.923464	102.591775	0.000001
	SOA	-0.053881	0.007635	-7.057022	0.000001
	Saliency	-8.719696	5.624365	-1.550343	0.121133
SUN ICA (2)	Constant	398.038248	3.881598	102.544934	0.000001
	SOA	-0.054030	0.007638	-7.074026	0.000001
	Saliency	0.364929	5.603101	0.065130	0.948074
SUN DOG (2)	Constant	397.135732	4.100411	96.852659	0.000001
	SOA	-0.053946	0.007641	-7.060189	0.000001
	Saliency	2.056810	5.751933	0.357586	0.720671
Bruce & Tsotos (2)	Constant	396.963804	4.087057	97.127052	0.000001
	SOA	-0.053984	0.007638	-7.068204	0.000001
	Saliency	2.381388	5.709937	0.417060	0.676655
Itti, Koch & Niebur (2)	Constant	396.235715	4.312019	91.891002	0.000001
	SOA	-0.054000	0.007637	-7.071269	0.000001
	Saliency	3.667551	6.114771	0.599786	0.548681
Zhai & Shah (3)	Constant	394.681650	3.510099	112.441734	0.000001
	SOA	-0.054239	0.007635	-7.103872	0.000001
	Saliency	8.222071	5.002286	1.643663	0.100319

Model (method)		β	Std. Error	t value	Pr(>|t|)
SUN ICA (3)	Constant	398.350576	3.524468	113.024309	0.000001
	SOA	-0.054045	0.007637	-7.076492	0.000001
	Saliency	-0.310456	4.972281	-0.062437	0.950218
SUN DOG (3)	Constant	392.503239	3.800210	103.284630	0.000001
	SOA	-0.053838	0.007633	-7.053486	0.000001
	Saliency	11.072075	5.034701	2.199152	0.027920 ***
Bruce & Tsotos (3)	Constant	394.591120	3.683474	107.124731	0.000001
	SOA	-0.053943	0.007635	-7.065408	0.000001
	Saliency	7.496456	5.010818	1.496054	0.134713
Itti, Koch & Niebur (3)	Constant	396.595948	3.234775	122.603870	0.000001
	SOA	-0.054006	0.007636	-7.072695	0.000001
	Saliency	4.675457	4.798386	0.974381	0.329922
Zhai & Shah (4)	Constant	393.760655	3.537627	111.306434	0.000001
	SOA	-0.053868	0.007633	-7.056902	0.000001
	Saliency	10.270012	5.060635	2.029392	0.042480 ***
SUN ICA (4)	Constant	397.833645	3.511731	113.287039	0.000001
	SOA	-0.054019	0.007637	-7.073006	0.000001
	Saliency	0.887398	5.006221	0.177259	0.859313
SUN DOG (4)	Constant	396.512307	3.796634	104.437851	0.000001
	SOA	-0.053936	0.007638	-7.061800	0.000001
	Saliency	3.384938	5.149956	0.657275	0.511039
Bruce & Tsotos (4)	Constant	397.597723	3.696890	107.549231	0.000001
	SOA	-0.054002	0.007638	-7.070286	0.000001
	Saliency	1.299525	5.140937	0.252780	0.800450
Itti, Koch & Niebur (4)	Constant	396.251191	3.389356	116.910450	0.000001
	SOA	-0.053964	0.007636	-7.067030	0.000001
	Saliency	4.851707	4.806551	1.009395	0.312842

Table 3. Sample results of linear regression. Model Zhai & Shah, Method 2, SOA = 75 ms

	β	Std. Error	t value	Pr(>|t|)
Constant	415.019703	5.163265	80.379322	0.000000 ***
Saliency	-17.271956	9.029303	-1.912878	0.055965 **

The dependency between reaction time and saliency value was noticed at a statistically significant level only for:

A – Zhai & Shah mode l, SOA = 75 ms, method 2 (p = 0.04) – Table 3
B – Zhai & Shah mode l, independently of SOA, method 4 (p = 0.06 – slightly above significance threshold) – Table 2
C – SUN DOG, independently of SOA, method 3 (p = 0.03) – Table 2

For case A (Table 3) the dependency was negative – a shorter reaction time for higher saliency was noticed. This result is consistent with the expectations. For cases B (Table 4) and C (Table 2) the dependency was positive. The results could induce that the Zhai & Shah and SUN DOG models better simulate the subject's behavior. To obtain conclusive results further experiments should be conducted.

Remarks

Analysis of the obtained results allowed to draw a few conclusions. Firstly, the influence of SOA on the response time was observed. This effect, however, was not consistent with expectations. It turned out that, regardless of the level of conspicuity on the locus where the stimulus appeared, for the shorter SOA subjects responded much more slowly. This results is independent of method used to estimate saliency. There may be several reasons for such situation but an exact solution would require further research. Based on information obtained from the subjects, it can be assumed that most of them for the SOA = 75 ms did not notice the stimulus appeared on the image. Some respondents argued that the stimulus appeared together with the picture and the response was difficult because there was no flicker effect, visible in the appearance of stimuli in the longer SOA. Perhaps the time of 75 ms was too short for respondents to prepare the reaction. Although attention has already made a move in the direction of the object, the subjects did not respond immediately

because they needed more time to program the motion. Koch, Itti and Niebur [1] assume 30-70ms time as sufficient to make any further saccades. The average time of fixation, however, is given as approximately 200 ms. Perhaps the respondents needed time to move gaze fixated on the point of concentration. It is also worth to remember that the technique based on the Posner task was originally applied with a very simple stimuli. In this paper the cue (image) was extremely complex and full of information. Perhaps such a complex stimuli requires more complex analysis and the analogy to the Posner's experiment is not straightforward.

For method 1, SOA 600ms subjects obtained shorter reaction times for regions with higher saliency. It can mean that 600 ms was enough time to process the picture and attention mechanisms connected with saliency appeared. Thus, the results were similar to expected ones.

For method 2, SOA 75 ms, the Zhai & Shah model, the expected results were obtained. It can show that this model, although is the simplest one, can make the better prediction. Maybe such level of complexity is enough to predict human attention movement during free viewing of pictures. On the other hand, for method 4, Zhah & Shai model and for method 2 SUN DOG model, in general, results show that the higher level saliency gets, the longer reaction times are. This is opposite to authors predictions and opposite to results described previously. The proposed methods of estimating saliency and assumptions about the order of regions in term of level of saliency may be far from ideal. This issues should be examined in future.

In the rest of the models there was no correlation between saliency and reaction time. In could be connected with the fact that the measurement of the reaction times is a complex task and the results are often noisy. Furthermore, the reaction time is prone to many distracters: tiredness, test conditions, time of the day etc. Therefore, a large amount of samples is required. The described experiment was performed on only 20 subjects and 300 images and should be treated as a preliminary study. It could be assumed that to obtain more conclusive results the experiment should be conducted on more subjects and an extended image database.

The proposed methods of calculating the saliency value in particular regions gave different results. However, they do not allow to differ models in terms of predictability of human behavior. It was impossible to compare the models by using the proposed methods as well. Therefore, this variable should be further examined and its, effectiveness should be estimated. However for Zhai & Shah, method 2 and method 4, SUN DOG method 3, there was a visible and significant difference in reaction time in term of saliency. This shows that it is really worth to repeat the experiment and the proposed schema can be valuable and useful for testing the efficiency and refinement of attention models.

Conclusion

In this study an alternative to the eye-tracking method of evaluating models of attention was presented. The obtained results indicate a potential usefulness of the modified cueing task for this type of research. This technique allowed to notice that there is a connection between SOA and reaction time. The results, especially for model Zhah & Shai, indicate that it is worth to repeat and continue the research. There is a noticeable correlation between saliency and reaction time. In the future the experiments should be continued on more subjects and a lager image database to reduce the influence of noise on the reaction times. Furthermore, other methods of calculating the saliency value in image regions could be examined. Finally, in subsequent studies the spatial resolution of the proposed method could be increased by multiplying the division of the analyzed images into rectangles. The division on 9 fields was a major simplification, which also could affect obtained results.

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DIFFICULTIES IN DEVELOPING ALGORITHMS FOR APPLICATION OF PHYSICAL METHODS OF TREATMENT OF DEPRESSIONS: ELECTROCONVULSIVE THERAPY AS WELL AS OTHER METHODS OF ELECTRIC AND MAGNETIC STIMULATION

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Abstract: Therapeutic algorithms constitute a kind of aid for selection of an appropriate method of treatment and its subsequent stages, with regard to different diseases in order to achieve the highest effectiveness of the therapy. The work describes premises and difficulties in development of therapeutic algorithms, with regard to a number of physical methods which are applied in psychiatry in treatment of depressive disorders, and which make use of the phenomena of magnetic or electric stimulation.
Keywords: ECT, physical treatments, therapy algorithms

Introduction

Clinical studies performed on large groups of patients as well as clinical observations allow for determination of the optimum methods of therapy. A therapeutic stepladder can be described, informing what forms of therapy should start the treatment in a given disease unit and how the treatment should progress at the subsequent stages if the first selected therapy proves ineffective or too little effective. At the second and further stages, the therapeutic stepladder may consist of several steps. This is the way to create algorithms of treatment, i.e., schemes of conduct allowing the physician to operationalize the therapeutic process [1].

Although their simple forms have been is use for several centuries, therapeutic algorithms are an achievement of modern medicine, which offers several different forms of therapy or medication, either in a non-invasive form (usually as pharmacotherapy) or as invasive methods (various kinds of surgical operations), ect., for any given disease unit. Thanks to experiments on animals and clinical studies it is now possible to assess both the effectiveness of a given method and its safety. Sometimes strengthening of the former feature (effectiveness) requires joint administration of several methods of therapy [2].

Unlike many other medical specializations, psychiatry offers a wide range of various methods of treatment of different mental disorders, which issues partly from their poly-etiological character. It has both biological and non-biological techniques at its disposal. The latter ones include various psychotherapeutic schools while among the biological ones we can distinguish pharmacotherapy, phototherapy and methods of magnetic as well as electric stimulation. The present work discusses the latest two of the methods listed above.

Historical trait

Methods of treatment reflect the present state of medical knowledge – mostly in the area of etiology. This is why for centuries the methods of treatment in psychiatry have been related with beliefs and knowledge (or, rather, ignorance) about the nature and origins of mental disorders, which, in fact, have been identified quite recently. In the 18th century, Giovanni Fantoni, a professor of the University in Turin, claimed that the structure of the brain is unknown, the disorders connected with it are inconceivable and its functions - impossible to comprehend (Obscura textura, obscuriore smorbi, functiones obscurissime) [3]. It was as late as the 19th century that the modern systems of classification of mental disorders taking into account nosological elements came into being. The beginning of the 20th century brought about new methods of therapy including shock therapies (including cardiazol and indoklon shocks as well as electroconvulsions), sleep therapy (prolonged sleep therapy, insulin coma therapy)

or fever therapies. The antidepressant and antipsychotic drugs that are used today were developed as late as the middle of the 20th century. Before that time, various herbal medicines including opiates, alcohol and hypericum extracts were used in treatment of mental disorders [4].

Since the Antiquity numerous physical methods using magnetic or electric phenomena have been used. Therapies tried to make use of the natural sources of these phenomena, like amber, magnetite or electric fish. In a later period, due to the progress of technology, medicine started to use Voltaic piles, electrostatic generators and various electric stimulators as well as artificial magnets and magnetic field generators [5, 6].

The best known method of physical treatment in psychiatry is electroconvulsive therapy (ECT) introduced into clinical practice in 1938 by Cerletti and Bini. They were the result of numerous and long-lasting attempts at using electric stimulation in therapy of mental disorders based on observations connected with chemically induced (indoklon, cardiazol) convulsions. Cerletti and Bini managed to select the current parameters that were able to trigger seizure activity of the brain [7]. It is interesting that the first application of ECT was preceded by several years of experiments of animals, which was then (in the 1940s) hardly ever practiced, and which is now an obligatory requirement of admission of new pharmacologically active substances for clinical studies [8].

Electroconvulsive therapy proved to be highly effective at controlling of both psychotic symptoms and affective disorders (in depression as well as in mania), making it possible for chronic patients to function fairly well in everyday life [9]. As compared to the chemical methods of inducing convulsions, ECY turned out to be safer and easier to administer. It was easier to control the dose of the stimulus (here: electric current) necessary to trigger a seizure. Moreover, convulsions were manifested immediately and not after a longer or shorter delay (in the case of chemically induced convulsions), during which the patient experienced discomfort.

Electroconvulsive technique was readily adopted in numerous psychiatric centers and soon became quite popular – initially applied larga manus in most psychiatric disorders.

The first two decades since the introduction of ECT into clinical practice were the period of intensive clinical studies, application of electroconvulsive procedures in various psychiatric disorders and accumulation of experience which allowed physicians to develop optimum (effective and safe) schemes of treatment, e.g. [8]:

– indications and counter-indications for application of ECT were developed (renouncing application of ECT in disorders in which it proved little effective),
– the regime of 4-15 procedures in a series was introduced, replacing mega-schemes (several dozen to several hundred procedures in a series, which could be highly dangerous and threatening with numerous side-effects),
– sine waves of the current were replaced by square waves (less burdening energetically),
– unilateral procedures were proposed in the place of bilateral ones (which, on the one hand, is safer as regards the risk of the occurrence of memory disorders and disturbances of other cognitive functions, but, on the other hand, is clinically less effective),
– application of pharmacologically modified procedures (short-term general anesthesia + muscle relaxation + oxygenation) decreased the risk of complication in the osseous system and cardiovascular system,
– monitoring of the seizure activity was introduced (seizures lasting at least 30 seconds were recognized as clinically effective, shorter ones were described as abortive – having no clinical effectiveness),
– appropriate legal mechanisms for application of ECT were developed (the procedure of obtaining the patient's informed consent was introduced).

The 1960s witnessed a certain „downfall" of ECT [10]. Three main reasons for this state of things can be discerned:

– Indications for application of ECT were seriously limited as a result of hitherto observations and studies indicating little effectiveness of ECT or its absence in nervous disorders, situational disturbances, personality disorders, etc.
– Modern antidepressant and antipsychotic drugs were introduced and popularized in clinical practice, assuming the role of first-stage treatment in most psychiatric disorders.
– Patient's rights movement developed, demanding drastic limitation of application of ECT and even completely rejecting psychiatry as a medical science (here the most important trend was the so-called anti-psychiatry).

In turn, in the recent decades we have had to do with a specific revival of ECT. Pharmacotherapy has not fulfilled the hopes pinned upon it. Its effectiveness amounting to 60-65% is recognized as lower than that of ECT (70-90%). There are still many patients for whom pharmacological therapy proves to be insufficiently effective and the intensity of ailments requires more invasive treatment including electroconvulsive therapy. Introduction of the mechanisms listed above allowed for significant improvement of safety of the therapy as well as the patient's comfort. Thus, so far, anti-psychiatry and its epigones have not managed to marginalize psychiatry or get rid of biological methods of psychiatric treatment (anti-psychiatry rejected not only ECT but also pharmacological therapy).

Present status quo

Electroconvulsive therapy is practically one of the old physical methods of psychiatric treatment, which managed to survive up today - mostly due to its high effectiveness and significant safety. More frequent use of ECT procedures is seriously limited by their unfavorable associations and the necessity of application in hospital conditions with the use of anesthetic techniques (general anesthesia and muscle relaxation) as well as the traditions of application of ECT in a given center and availability of ECT equipment. Moreover, as it has already been mentioned, mental disorders of lesser intensity or of situational etiology respond fairly well to pharmacological treatment in outpatient clinics. Hence, prescribing of ECT in this kind of cases would be unjustified.

Also, numerous more severe cases of mental disorders respond fairly well to intensive pharmacotherapy applied in hospitals, so there is no need to apply ECT procedures.

Due to its high safety, good availability, easy administration and low cost, pharmacological treatment is commonly recognized as the first-choice therapy. Patients suffering from mental disor-

ders should not be afraid that psychiatrists will direct them a priori for electroconvulsive therapy. On the other hand, the decision about administration of ECT should not be made on a short-term basis, and the ECT procedures ought to constitute an element of the algorithm/plan of therapy, which should include a discussion of the course of the therapy with the patient (which usually improves their compliance), counteract irrational anxiety, and prevent the situation in which the decision about administration of ECT would be made late/too late or even renounced. In the case of the patients younger than 19 or those whose health condition makes it impossible to issue informed consent, it is the regional family court that should approve of application of ECT [11].

Pharmacological treatment itself usually consists of several phases including mono- and poly pharmacotherapy, also using potentiating/augmentative drugs, etc. Moreover, treatment of "ordinary" depressive disorders (e.g. recurrent depressive disorders in a unipolar disorder) differs from that in the course of depression in bipolar affective disorder (where manic episodes occur and a mania/depression switch might take place) and in psychotic depression (delusional depression, schizoactive disturbances), which require different selections of drugs [12].

ECT is usually placed at the remotest stage of the therapeutic stepladder. In the monograph by Grunze and Walden [13] we can find several algorithms of therapy of affective disorders. For instance, in the therapy of a depressive episode in the course of bipolar affective disorder application of ECT procedures is supposed to take place at the fourth stage. A similar place is assigned for ECT in the algorithm suggested for treatment of schizophrenia. In the case of an acute episode of euphoric mania or a mixed episode ECT are placed at the third stage of the therapeutic stepladder.

The so-called Texan Algorithm of treatment of affective disorders is even more complex [14]. In an episode of major depression it is the presence or absence of psychotic symptoms that determines the stage at which ECT is to be administered. While in the latter case ECT is placed at the sixth stage of the therapeutic stepladder, the occurrence of psychotic symptoms makes administration of ECT more urgent and allows for it as early as the third stage.

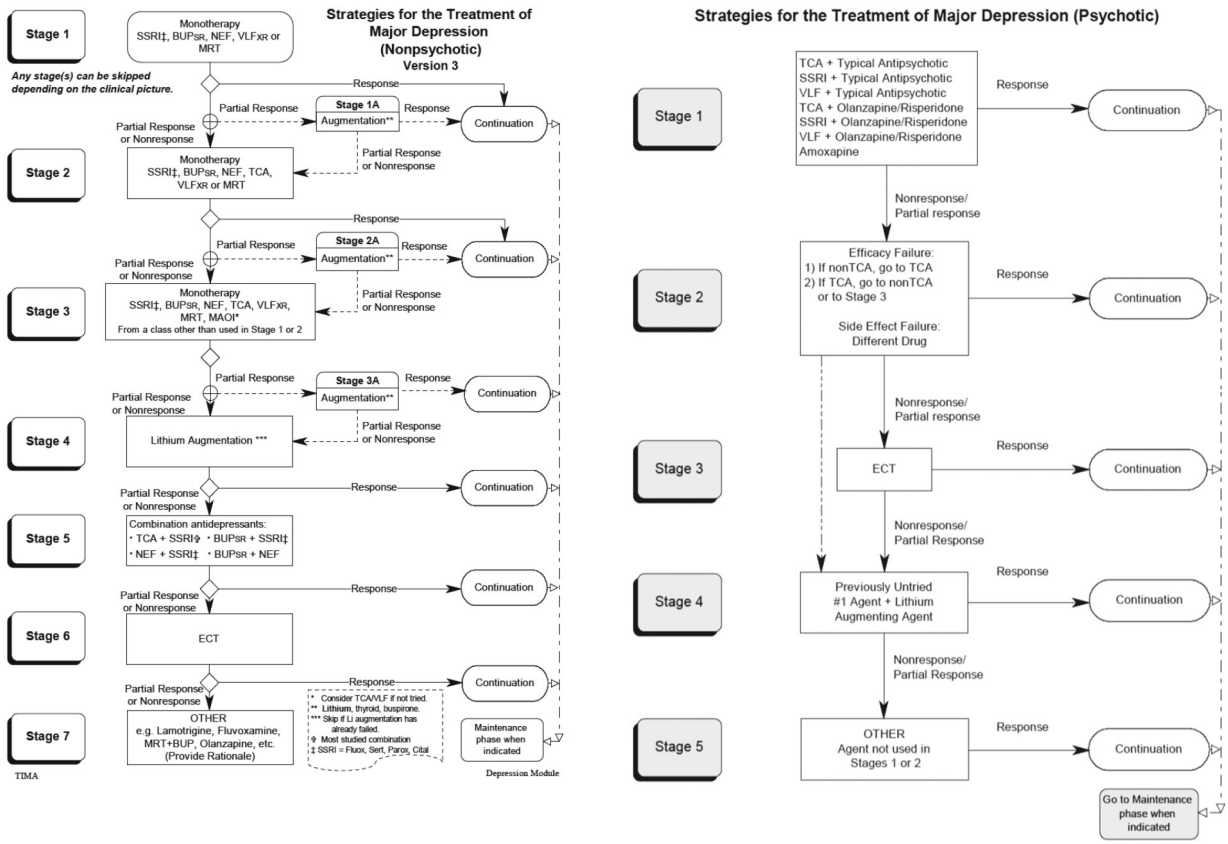

Fig. 1. Algorithms of psychiatric therapy in major depression without psychotic symptoms (left) and with them (right) – placement of ECT therapy [14]

However, strict adherence to the prescribed periods of time necessary to check the effectiveness of every stage of the therapeutic stepladder (1 month) might cause that the patient (case of non-psychotic depression) would be subjected to ECT only after half a year of testing various subsequent forms of pharmacotherapy and suffering from usually rather acute clinical symptoms of depressive disorder (including a high hazard of suicide). Hence, therapeutic algorithms have their own rules but clinical experience causes that in definite cases it is appropriate and recommended to make the decision concerning administration of ECT to a patient much earlier.

However, in definite cases it is allowed to administer ECT as the first-choice method, or, in other words, at the first stage of the therapeutic stepladder. Polish standards and algorithms of

the therapy of affective disorders developed in 1998 by a team of experts supervised by the Institute of Psychiatry and Neurology in Warsaw and the National Health Consulting and Control Board [15] recommend administration of ECT as the first-stage method in the following situations:

– depressive state with intensified suicidal tendencies (making effective prevention of a suicidal attempt impossible) and depression constituting a direct threat to the patient's life due to rejection of food (e.g. in depressive stupor);
– health condition rendering administration of antidepressant drugs impossible;
– persistent drug-resistant depressive states of at least moderate intensity, treated for at least 6 months (this case, however, can hardly be recognized as recommendation of ECT as the first-choice method if it is to be preceded by half a year of pharmacological treatment).

In its report of 2001 [16], the American Psychiatric Association (APA) proposed the following indications (different and more comprehensive than the Polish ones) for administration of ECT as the first stage method:

– a need for rapid, definitive response because of the severity of a psychiatric or medical condition,
– when the risk of other treatments outweighs the risk of ECT,
– a history of poor medication response or good ECY response in one or more previous episodes of illness,
– the patient's preference.

As can be seen, American directives do not refer to definite disease units but to the severity of the health condition. Situations like: drug resistance, manifestation of pharmacotherapy induced side effects of higher intensity than that expected after ECT as well as deterioration of the psychiatric or somatic condition requiring a fast and effective therapy are, according to the APA, second-choice criteria.

The latest of the indications given by the APA, the one confirming the patient's subjective role in the therapy, seems interesting. Thanks to it the patient whose health improved during the previous episode of the disorder due to ECT has the right to demand its administration at a much earlier stage of the therapy during the subsequent recurrence of the disorder. Obviously, this also creates a possibility of a reverse situation - it would be very hard to administer ECT procedures if the patient refused to accept them. Although, theoretically, it is possible to ask an expert physician of the local Family Court to issue a permission of administration of ECT without the patient's consent, actually it would be very difficult to execute (the necessity of direct coercion would disturb the relation between the patient and the physician significantly). It would be much better to work on the patient's motivation in order to obtain their informed consent to ECT.

Therapeutic algorithms have their definite value that allows, among others, for administration of the preceding pharmacological treatment in an appropriate way (i.e., using a proper dose for a correct period of time). On the other hand, it should be stated that the treatment with ECT must never issue from a mechanical interpretation of the recommended algorithms. The authors of the APA Report clearly state: ECT as a therapy, with well defined indications, should not be reserved for use as a "last resort". Such practice may deprive patients of effective treatment, postpone recovery and prolong suffering as well as contribute to developing of drug resistance" [16].

Apart from the above mentioned, there exist other Polish and foreign algorithms of application of electroconvulsive therapy.

The above discussion shows that as regards therapeutic decision making (concerning administration of ECT), the following elements, some of which have not been mentioned before, must be taken into account:

– diagnosis
⇨ ECT is recommended mostly for the patients suffering from affective disorders and schizophrenia, especially in its catatonic form;
– course of illness and the present intensity of symptoms
⇨ at least moderate intensity of symptoms, drug resistance, severe somatic condition, life-threatening condition;
– special populations of patients
⇨ due to the high safety of the procedures as well as their local and not general action, benefits of application of ECT may be particularly significant, in particular in elderly patients and pregnant women; on the other hand, these procedures are not administered to children and in adolescents they are administered very seldom in particularly hard situations;
– the patient's consent
⇨ ECT is mostly administered to the patients who have consented to application of this form of therapy; if the patient's condition does not allow for issuing such a consent, the Family Court is applied for acceptance of this treatment;
– special circumstances
⇨ ECT procedures are generally executed in hospital conditions; the hospital must possess appropriate equipment (apart from ECT apparatus it is recommended to have a cardiomonitor, pulse oximeter and anestesiological equipment); in definite conditions (ultima ratio) it is possible to administer non-modified ECT (without general anesthesia and muscle relaxation; the author knows Polish centers where procedures of this kind were executed not long ago);
– cost
⇨ ECT treatment is obviously more expensive than pharmacotherapy; it is necessary to purchase/obtain appropriate equipment (see above), employ an anesthesiologist and, sometimes, an anesthesiological nurse, purchase proper drugs and materials which are hardly ever used in classical psychiatry, executing additional examination and paying for specialist consulting necessary in the process of qualifying a patient for ECT. The above cost is not refunded by Polish National Health Fund; this is why certain big regional hospitals do not offer ECT procedures. It seems that Polish health system neglects the so-called economics of the process of treatment whose objective is to make the patient's therapy the shortest and the improvement as good as possible;
– attitudes of the personnel
⇨ determine how fast the decision of administering ECT is made and what proportion of patients receive it; the so-called discretionary element seems to play rather an important role as regards administration of ECT in a given psychiatric center, etc.

While developing therapeutic algorithms it would be necessary to assign definite significance to the issues listed above. This, however, seems to be a very complex task.

New methods of electric and magnetic stimulation

During the last two decades, several new physical methods were subjected to clinical studies [17, 18]:

- transcranial magnetic stimulation TMS,
- magnetic seizure therapy/magnetic convulsive therapy MST/MCT,
- vagus nerve stimulation VNS,
- deep brain stimulation DBS, and
- transcranial direct current stimulation tDCS.

Among the above mentioned physical methods only the vagus nerve stimulation VNS has been accepted for administration in treatment of chronic, drug-resistant, recursive depressive disorders. The remaining methods are recognized as experimental and their administration is of cognitive rather than regular character - therefore they are not included in therapeutic algorithms. Moreover, their actual effectiveness seems relatively low.

In the case of TMS, its effectiveness could be increased by application of the very expensive and hardly accessible methods of functional neuroimaging (in order to identify the metabolically disturbed structure of the brain) and neuronavigation (that allows for application of magnetic stimulation precisely over the disturbed area of the brain).

Thus, there is little sense in evoking seizure activity with the help of magnetic field (which is a very hard task due to the high current parameters of TMS stimulators) instead of using electric stimulation, namely, ECT. The potentially higher safety of the magnetic method is negligible in the face of its poorly investigated (and probably lower than in ECT) effectiveness.

The current parameters applied in tDSC are so low (a few Volt of direct current applied to the head) that the actual effectiveness of this method seems illusory and offering of such a method - unethical.

VNS and DBS methods are the most invasive techniques currently used in psychiatry: they require a surgical operation on the peripheral or central nervous system. They are also (like TMS and MST/MCT) exceptionally expensive, which limits their application to a small number of centers in the world.

Literature studies have allowed for identification of certain contradiction in practical application of VNS. Due to its highly invasive character it should be applied as the last choice method. However, clinical investigations have shown that VNS is most effective in treatment of mild and moderate forms of depressive disorders [19]. These, however, can well be treated with pharmacotherapy and it is hard to imagine a patient with moderate symptoms of the disorder who can receive effective pharmacological treatment and who would be ready to submit to an invasive and hardly reversible procedure of implantation of a VNS stimulator.

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POTENTIAL APPLICATIONS OF CELLULAR PHONE SYSTEMS FOR MEASURING AND MODELING TOURIST MOVEMENT

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Abstract: Measuring the volume and describing the structure of tourist movement seems be one of the crucial tasks of subjects responsible for creating and executing tourism policy. Information on the above-mentioned parameters constitutes the fundamental factor for tourism management at all levels of state administration (i.e. national, regional, local). Its importance manifests itself not only in current activities but also (or possibly foremost) in programming and planning tourism development. What seems to be of crucial importance in both latter processes is creating a proper volume, as well as dimensional, temporal and generic structure, optimal for a particular area (i.e. a city, county, province, country). Generating and executing tourism policy in different spatial arrangements, in particular modeling tourist movement, requires reliable data. Meanwhile, insofar applied methods of statistical registration or other related ways of measuring tourist movement prove imperfect, hence affecting negatively the quality of tourism planning. As a matter of fact, planning activities in tourism sector are frequently criticized both in the countries with highly developed tourism and those still aspiring to such a high-profile status. The paper presents basic assumptions of Telephone System for Cellular Analysis of Tourist Movement (TelSKART©), which is an innovative method of measuring the volume of tourist movement, using second and third generation of cellular phone technology (i.e. *GSM – Global System for Mobile Communications, and UMTS – Universal Mobile Telecommunications System*).
Keywords: tourist movement, measurement, TelSKART©, mobile phones, GSM.

Information Communication Technologies (ICTs) have been transforming contemporary tourism industry. The ICT driven reengineering has gradually generated a new paradigm-shift altering the industry structure and developing a whole range of opportunities and threats [4]. It concerns also to tourism research, especially to tourism geography – in field of measuring and modeling of tourist movement. According to J. Xia and C. Arrowsmith, spatio-temporal modeling of tourists movements considers how people move about or why exhibit certain movement behaviours. Research into spatio-temporal movements of tourists can be studied from a number of different aspects. Psychologists, for example, are concerned with understanding the cognitive aspects of why people move along particular pathways in preference to alternative pathways. Geographers and tourism researchers are more interested in how people move around particular locations (tourist regions, destinations) and model what is observed in a visitor movement [10].

As is widely known, measuring the quantity of tourist movement is an eternal dilemma in tourism research, and thus far no one has developed an effective method of solving it. Ways of statistically registering tourist movement to date have proven inadequate, as have other methods of measurement, and estimates acquired on their basis can vary by up to several hundred per cent, as many researchers in Poland and abroad have demonstrated. The basic data on tourist movement is supplied by the "hotel method", based on information taken from accommodation establishments. This allows us to define the number of people using accommodation establishments covered by mandatory guest registration, by consulting the KT-1 statisticalforms. Unfortunately, this method gives us no chance to define the total number of people temporarily staying in a given area, as some of them use no accommodations at all, or sleep in places which slip out from under the official statistics (apartments of friends and relatives, "second homes", private rooms etc.). We must also bear in mind that some portion of accommodations fall under the heading of "gray economy". Therefore, data produced on the basis of hotel registrations is quite inexact and sometimes misses a full fifty per cent of the number of tourists staying in a given area. Other methods (e.g. research done in sites offering tourist services, institutions managing tourist attractions, and various sorts of indirect methods, including: passenger transport statistics, tickets to events, national parks, the beach,

resort payments, or the "M. Boyer baked-goods" method have also turned out to be ineffective.

A fine example of the problems in measuring the quantity of tourist movement is Zakopane, where the city authorities and sites responsible for tourism development (the Promotional Bureau) confess that "*…in fact, no one knows how many tourists come to the Tatra Mountains during high season. The estimates vary greatly – some calculate that in August there were around half a million people, of whom the majority visited Zakopane, while others reckon that there could only have been around 80,000 tourists in the city itself*" [3]. This view is shared by the Mayor of Zakopane, who – well aware of how important it is for the tourist movement organization to know how many tourists and visitors are in the town and the region – announced (in an interview of September 11th of this year) that in 2010 the town would be counting the number of incoming tourists. He wants to supplement the traditionally applied, though imperfect hotel method with a tourist count, using a few dozen trained volunteers and the model of counting visitors to a national park [11]. For a town this is significantly more difficult, however, as tourists go by various routes, and not along marked trails, as they do in the national parks.

The idea of using the mobile telephone system to research tourist movement was born after reading a report on the current situation of the telecommunications market in Poland, according to which practically every statistical Pole possesses a mobile phone (over 41 million mobile phone devices are presently registered in Poland) [12]. Having side by side the information that practically every Pole has a mobile phone, and the difficulties encountered in measuring tourist movement, we can realize that almost every tourist has such a telephone handy, and the majority of tourists will use it at least once, to share their impressions of the trip, to ask what's happening at home, to arrange some important business etc. The main problem was: if there existed the technological possibilities to define the number of calls coming from tourist sites over the course of a day, a month, or a year (it later turned out that it wasn't even necessary to call for an operating device to be registered by the base station). Even if it wasn't possible to discriminate the number of telephones owned by tourists, a simple subtraction of the town's permanent residents from the total number of telephones used in a given period allows us to make a fairly exact – compared to other methods to date – definition of the number of visitors.

The next step of activity for creating new method of researching tourist movement was a crash course in the principles of how second (GMS) and third (UMTS) generation mobile phones operate, with a particular focus on the technology and functioning side of making and recording calls.

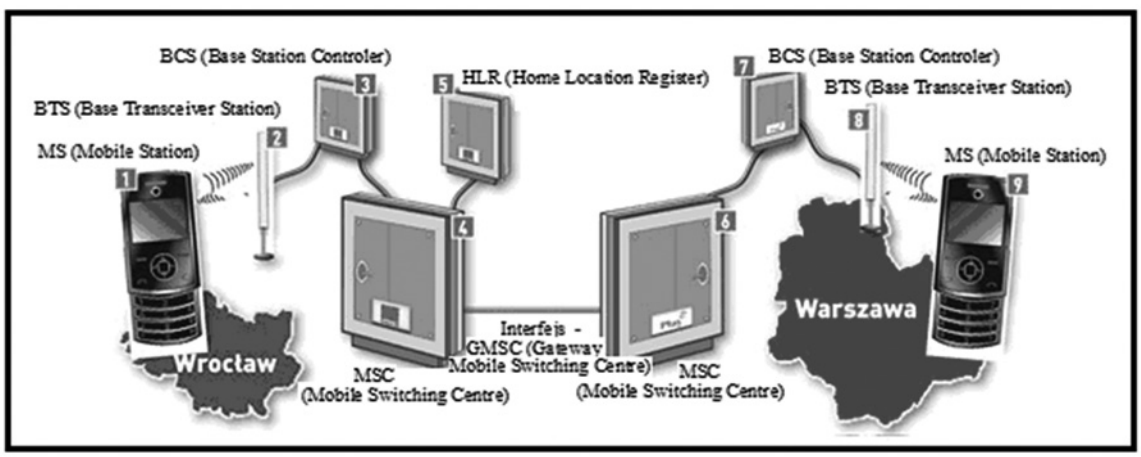

Fig. 1. Schematic view of the mobile telephone system (GSM)

Source: Paczuski T., Jak działają sieci komórkowe?, [in:] Komputer Świat of 30 September 2009 – http://www.komputerswiat.pl/jak-to-dziala/2008/02/sieci-komorkowe.aspx (7.11.2009)

Apart from reading the relevant literature, principles of the method were also consulted by experts in the telecommunications field, particularly workers in the State Communications Institute in Warsaw and the AGH University of Science and Technology (Faculty of Electrical Engineering, Automatics, IT and Electronics) and one of four Polish mobile telephone operators). These consultations indicated that we have the technological capacity to define the number of mobile telephones working at a chosen time, in a chosen, defined area equipped with base stations (at the moment these cover practically the entire map of Poland) [8].

Having gathered this information, we can begin to create the basis for a new method of researching tourist movement, which I have named TelSKART©, short for Telephone System for Cellular Analysis of Tourist Movement. The method is based on the analysis of radio signals that are captured from tourists' cellular phones by base stations located in regions of tourist reception. A basic element of the GMS network system, and also a basic unit in analyzing tourist movement in the TelSKART© method, are the cells. I would like to emphasize, however, that in the professional telecommunications terminology – unlike in colloquial speech – this "cell" is not the device from which we

phone (which carries the name "mobile station") – but the area covered by a single base station. We might say half in jest, then, that what gentlemen generally carry in their pockets and women in their purses is – in professional terms – not a cellular, but a mobile station. Every mobile phone that is switched on keeps in steady contact with the antennae of the base stations whose signals it receives best. If the signal grows weak – while driving a car, for example – the base station controller automatically directs the link to the nearest station (antenna). The cellular network always knows which antenna our telephone is using, and thus where we are at any given moment.

With the TelSKART© method we will be able to count tourists on the basis of their running mobile stations (or mobile phones), which will be registered by base stations (antennae). Information on how many of those mobile stations (in tourists' pockets, purses and backpacks) were located in a given area at a selected period of time – after processing and inscribing in the relevant registers (HLR and VLR) – can be acquired through Operation and Maintenance Center (OMC) networks. The whole procedure of making calls is obviously more complicated, and is made up of various elements. The architecture of the GSM system can be illustrated by the ideogram below, in simplified form.

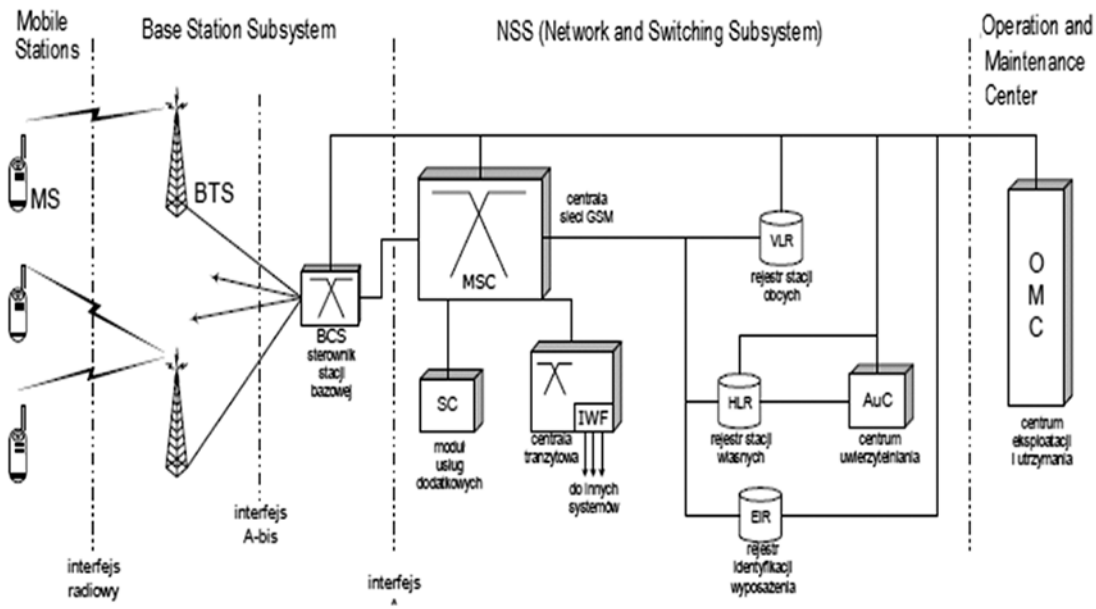

Fig. 2. The Architecture of the GMS System

Legend: MS (Mobile Station); BTS (Base Transceiver Station); BCS (Base Station Controller); MSC (Mobile Switching Centre); GMSC – Gateway Mobile Switching Centre); IWF (InterWorking Functions); HLR (Home Location Register); VLR (Visitor Location Register); AuC (Authentication Centre); EIR (Equipment Identity Register); OMC (Operation and Maintenance Centre); Operation and Maintenance Subsystem OMS); NSS (Network and Switching Subsystem)

Source: Kozłowska A., Communications systems, pp. 1-4. Work published at: http://www.stud.pwr.wroc.pl/Stare/Systemy_tel/wyklad_2_1.pdf (18.12.2009)

What are the basic premises and advantages of the TelSKART©? This method:

- assumes that the quantity of tourist movement (number of tourists) staying at a given time in a freely defined area of tourist reception (particular locales, communities, districts, townships, and even countries, in the case of foreign tourists);
- it allows us to define not just the quantity, but also freely defined spatial structures and seasonal changes in tourist movement, with freely selected periods of analysis time (even down to certain hours);
- can be of major significance for the quality development of tourist movement research, facilitating the optimal selection of research samples for quality analyses (e.g. surveys).

There are doubtless other possible uses of this technology – e.g. researching tourist itinerary routes (streams of tourist movement), based on changing telephone locations, identified by consecutive base stations. It would seem that the method presented here creates very fine conditions for researching various aspects of tourist movement. And yet, its primary advantage is that it will help us to define the amount of tourist movement in localities and regions with a high degree of accuracy.

The method is still undergoing development. It has to be fine tuned from a "tourist" perspective, in other words, the needs and capabilities of information gathering have to be established,

especially in terms of acquiring information more complex than simply the number of tourists (e.g. dynamics, spatial structure and type of tourist movement, perhaps even the identification of tourist itinerary paths, attractions visited, and even more particularly defined behavior of tourist excursions). The technical details also need to be ironed out, creating programs to sort out selected data held by cellular network operators, and above all, to win over the cooperation of these operators. It would seem, however, that the matter is important enough to overcome the problems involved. Some difficulties could be created by the protection of personal data, as pointed out by some commentaries to my conference presentation. But it seems that this is not the most important issue, as for our purposes – the identification of the overall number of tourists staying in a given area – we needn't personalize the data acquired from the operators, as it is only the general count of registered telephone devices that interests us, and not information regarding specific numbers, much less their owners.

In the near future TelSKART© will be tested in Zakopane. It seems that the implementation of this method should be of interest to not only local governments and local and regional tourist agencies, but also representatives of various sectors of the tourist industry. Above all, however, it seems that this method ought to interest the most important institutions responsible for the development of tourism in Poland, i.e. the Ministry of Sport and Tourism and the Polish Tourist Organization. This method has one more great advantage: the enormous amount of money it could save. The data we need is already collected in the relevant operator registries. It only needs to be knowledgeably extracted and correctly used. The money conserved through the use of this method could be spent on quality analyses of tourist movement.

It seems that using modern technologies in tourist movement research creates opportunities we might have only dreamed about until recently. The method presented is a good example of this. Its spread and implementation has one more very important aspect. This is the fact that Poland could be a pioneer in this kind of research. The initial analyses of the professional literature (including both traditional academic journals and Internet resources) have shown that, to date, GSM cellular phone systems have probably never been used anywhere in the world to measure the global quantity and structure of tourist movement. There are only a few works that have used GSM for analysis of foreign tourism (in Estonia) and works that have used GPS (Global Positioning System), part of the equipment of the latest telephone devices (for the time being only a minimal percentage of cellular phones have this). Research has been conducted in which the position of specific tourists was established through the GPS installed in telephones, particularly in crisis situations, such as when a tourist has been lost in the mountains.

To conclude, we ought to add that apart from measuring tourists, this method creates a splendid opportunity to conduct quality research on tourist consumption (e.g. through a detailed, very representative method of choosing tests for tourist survey research – it would suffice to invite every tenth or hundredth tourist to take part – according to the laws of high numbers – to acquire quite a representative research sample). It is also worth mentioning that the system of third-generation cellular telephones (UMTS) creates even better conditions for using the TelSKART©method in tourist movement research.

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The method TelSKART© was presented for the first time during the conference *Regional Research on Tourist Services Consumers*, organized by the Polish Tourist Organization and the Ministry of Sport and Tourism (November 24-25 in Warsaw. Work published at: http://www.pot.gov.pl/dokumenty/do-pobrania/materia142y-szkoleniowe/Program%20konferencji%2024_25_11_2009.pdf/

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A COMBINED SVM-RDA CLASSIFIER FOR PROTEIN FOLD RECOGNITION

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Abstract: Predicting the three-dimensional (3D) structure of a protein is a key problem in molecular biology. It is also an interesting issue for statistical methods recognition. There are many approaches to this problem considering discriminative and generative classifiers. In this paper a classifier combining the well-known support vector machine (SVM) classifier with regularized discriminant analysis (RDA) classifier is presented. It is used on a real world data set. The obtained results are promising improving previously published methods.

Keywords: protein fold recognition, support vector machine, multi-class classifier, one-versus-one strategy

Introduction

Predicting the three-dimensional (3D) structure of a protein is a key problem in molecular biology. Proteins manifest their function through these structures, so it is very important to know not only sequence of amino acids in a protein molecule, but also how this sequence is folded. The successful completion of many genome-sequencing projects has meant that the number of proteins with known amino acids sequence is quickly increasing, but the number of proteins with known 3D structure is still relatively very small.

There is a variety of different aproaches to the protein structure prediction. They range from those based on physical principles, through methods that rely on evolutionary information, to the statistical methods based on machine-learning systems. An interesting survey of these methods can be found in Rychlewski et al. [22]. In this paper we focused on machine-learning algorithms (Stąpor [20]).

There are several machine-learning methods to predict the protein folds from amino acids sequences proposed in literature. Ding and Dubchak [5] experiment with support vector machine (SVM) and neural network (NN) classifiers. Shen and Chou [9] proposed ensemble model based on nearest neighbour. A modified nearest neighbour algorithm called K-local hyperplane (HKNN) was used by Okun [14]. Nanni [13] proposed ensemble of classifiers: Fisher's linear classifier and HKNN classifier.

There are two standard approaches to the classification task: generative classifiers use training data to estimate the probability model for each class and then test items are classified by comparing their probabilities under these models. The discriminative classifiers try to find the optimal frontiers between classes based on all the samples of the training data set.

This paper presents a classifier, which combines the support vector machine (SVM) – discriminative classifier – with the statistical regularized discriminant analysis (RDA) – generative classifier. The SVM technique has been used in different application domains and has outperformed the traditional techniques. However, the SVM is a binary classifier but the protein fold recognition is a multi-class problem and how to effectively extend a binary to the multi-class classifier case is still an ongoing research problem. There are many methods proposed to deal with this issue

One of the first and well-known methods is one-versus-one strategy with max-win voting scheme. In this strategy all binary classifiers vote for the preferred class. Originally a class with the maximum number of votes is recognized as the correct class.

However, some of these binary classifiers are unreliable. The votes from these classifiers influence the final classification result. In this paper there is a strategy presented to assign a weight (which can be treated as a measure of reliability) to each vote based on the values of the discriminant function from an RDA classifier.

The rest of this paper is organized as follows: Section 2 introduces the database and the feature vectors used is these experiments, Section 3 presents the basis of RDA classifier, Section 4 shortly describes basics of the SVM classifier, Section 5 describes the method of combining the classifiers and Section 6 presents experimental results and conclusions.

The database and feature vectors

Using machine-learning methods entails the necessity to find out databases with representation of known protein sequences and its folds. Then this information must be converted to the feature space representation.

Database

In experiments described in this paper two data sets derived from SCOP (Structural Classification of Proteins) database are used. The detailed description of these sets can be found in Ding and Dubchak [5]. The training set consists of 313 protein sequences and the testing set consists of 385 protein sequences. These data sets include proteins from 27 most populated different classes (protein folds) representing all major structural classes: α, β, α/β, and α + β. Where α are those whose structure is essentially formed by α-helices, β are those whose structure is essentially formed by β-sheets, α/β are those with α-helices and β-strands and α + b are those in which α-helices and β-strands are largely segrega-ted.

The training set was based on PDB_select sets (Hobohm et al. [18], Hobohm and Sander [19]) where two proteins have no more than 35% of the sequence identity. The testing set was based on PDB-40D set developed by Lo Conte et al. [8] from which representatives of the same 27 largest folds are selected. The proteins that had higher than 35% identity with the proteins of the training set are removed from the testing set.

Table 1. The protein folds used in experiments

Fold name	Structural class	Fold index	Number of proteins in	
			training set	testing set
Globin-like	α	1	13	6
Cytochrome c	α	7	7	9
Dna-binding 3-helical bundle	α	4	12	20
4-Helical up-and-down bundle	α	7	7	8
4-Helical cytokines	α	9	9	9
Alpha; ef-hand	α	11	7	9
Immunoglobulin-likeβ-sandwich	β	20	30	44
Cupredoxins	β	23	9	12
Viral coat and capsid proteins	β	26	16	12
Cona-like lectins/glucanases	β	30	7	6
Sh-3 like barrel	β	31	8	8
Ob-fold	β	32	13	19
Trefoil	β	33	8	4
Trypsin-like serine proteases	β	35	9	4
Lipocalins	β	39	9	7
(Tim)-barrel	α / β	46	29	48
Fad (also nad)-binding motif	α / β	47	11	12
Flavodoxin like	α / β	48	11	13
Nad(p)-binding rossman fold	α / β	51	13	27
P-loop containing nucleotide	α / β	54	10	12
Thioredoxin-like	α / β	57	9	8
Ribonuclease h-like motif	α / β	59	10	14
Hydrolases	α / β	62	11	7
Periplasmic binding protein-like	α / β	69	11	4
-Grasp	α + β	72	7	8
Ferredoxin-like	α + β	87	13	27
Small inhibitors, toxins, lectins	α + β	110	14	27
	Total		313	385

Feature Vectors

In these experiments, the feature vectors developed by Ding and Dubchak [5] were used. These feature vectors are based on six parameters: Amino acids composition (C), predicted secondary structure (S), Hydrophobity (H), Normalized van der Waals volume (V), Polarity (P) and Polarizability (Z). Each parameter corresponds to 21 features, except Amino acids composition (C), which corresponds to 20 features. The data sets including these feature vectors are available at http://ranger.uta.edu/~chqding/protein/. For more concrete details see Dubchak et al. [6], [7]. In this study the sequence length was added to the Amino acids composition (C) vector and the feature vectors based on these parameters were used in the different combinations creating vectors from 21D to 126D.

Statistical classifiers

Quadratic discriminant analysis (QDA) models the likelihood of a class as a Gaussian distribution and then uses the posterior distributions to estimate the class for a given test vector. This approach leads to discriminant function:

$$d_k(x) = (x-\mu_k)^T \Sigma_k^{-1} (x-\mu_k) + \log|\Sigma_k| - 2\log p(k) \quad (1)$$

where x is a test vector, μ_k is a mean vector, Σ_k is a covariance matrix and $p(k)$ is prior probability of the class k. The Gaussian parameters for each class can be estimated from training vectors, so the values of Σ_k and μ_k are replaced in the formula (1) by its estimates $\hat{\Sigma}_k$ and $\hat{\mu}_k$.

However, when the number of the training samples n is small compared to the number of dimensions of the training vector, the covariance estimation can be ill-posed. The approach to

resolve the ill-posed estimation is to regularize a covariance matrix. First, the covariance matrix Σ_k can be replaced by their average (pooled covariance matrix), i.e. $\hat{\Sigma} = \sum \hat{\Sigma}_k / \sum N_k$ which leads to linear discriminant analysis (LDA). This assumes that all covariance matrices are similar. It is very limited approach, so in regularized discriminant analysis (RDA) each covariance matrix can be estimated as:

$$\hat{\Sigma}_k(\lambda) = (1-\lambda)\hat{\Sigma}_k + \lambda\hat{\Sigma} \quad (2)$$

where $0 \le \lambda \le 1$. The parameter λ controls the degree of shrinkage of the individual class covariance matrix estimate toward the pooled estimate.

There is no universal value of λ parameter for all classification problems. This value must be experimentally chosen using cross-validation procedure on the training data set.

There can be further regularization using another parameter as follows:

$$\hat{\Sigma}_k(\lambda\lambda, \gamma) = (1-\gamma)\hat{\Sigma}_k(\lambda\lambda) + \gamma\, tr[\hat{\Sigma}_k(\lambda\lambda)]\, I / N \quad (3)$$

where $0 \le \gamma \le 1$, $tr[\hat{\Sigma}_k(\lambda\lambda)]$ is sum of eigenvalues of $\hat{\Sigma}_k(\lambda\lambda$. The parameter γ controls the degree of the shrinkage towards multiple of identity matrix for a given value of λ.

The SVM classifier

The support vector machine (SVM) is a well known large margin classifier proposed by Vapnik [10]. This technique has been used in different application domains and has outperformed the traditional techniques. The basic concept behind the SVM classifier is to search an optimal separating hyperplane, which separates two classes. However the perfect separation is not often feasible, so slack variables $\xi_1, \xi_2, \ldots, \xi_n$ can be used to measure the amount of the violation of the original constraints. Let us consider a classifier whose decision function is given by:

$$f(x) = sign(x^T w + b) \quad (4)$$

where x denotes a feature vector and w is a weight vector. Then the SVM algorithm minimizes the objective function:

$$\tfrac{1}{2}\|w\|_2^2 + c\sum_{i=1}^{n}\xi_i \quad (5)$$

subject to: $y_i(wx_i + b) \ge 1 - \xi_i,\ \xi_i > 0,\ i = 1, \ldots, n$

This problem leads to so called dual optimization problem and finally (considering non-linear decision hyperplane and using the kernel trick) to:

$$f(x) = sign\left(\sum_{i=1}^{N}\alpha_i y_i K(x_i, x) + b\right) \quad (6)$$

where $0 \le \alpha_i \le C,\ i = 1,2,\ldots, N$ are nonnegative Lagrange multipliers, x_i are the support vectors, x_i is a cost parameter that controls the trade off between allowing training errors and forcing rigid margins and $K(x_i, x)$ is the kernel function.

The proposed combined classifier

The SVM is a binary classifier but the protein fold recognition is a multi-class problem. There are many methods proposed to deal with this issue. In our classifier we use the first and well-known method: one-versus-one strategy with max-win voting scheme.

The well-known LIBSVM library version 2.89 was used in our research (Chang and Lin, [3]). Although the implementation of this library includes one-versus-one strategy for the multi category problems, only the binary version of the classifier was used. LIBSVM provides a choice of built-in kernels i.e. Linear, Polynomial, Radial Basis Function (RBF) and Gaussian. The RBF kernel:

$$K(x_i, x) = -y\|x - x_i\|^2,\ y > 0 \quad (7)$$

gave the best results in our experiments.

The parameters C from formula (5) and y must be chosen to use the SVM classifier with RBF kernel. It is not known beforehand which C and y are the best for the given problem. Both values must be experimentally chosen, which was done by using cross-validation procedure on the training data set. The best recognition ratio 58.7% was achieved using parameter values y=0.1 and C=128.

In one-versus-one strategy with max-win voting scheme the binary classifiers are trained between all the possible pairs of the classes. Every binary classifier votes for the preferred class and in this way the voting table is created. Originally a class with the maximum number of votes is recognized as the correct class.

However, some of these binary classifiers are unreliable. The votes from these classifiers influence the final classification result. In our method there is a strategy proposed to assign a weight (which can be treated as a measure of reliability) to each vote. The weight is based on the values of the discriminant function from an RDA classifier as described below.

In our experiments we decide to use selection algorithm to find better feature vector for an RDA classifier. The best selection method is to check all possible feature combinations. However, the number of them is far to high to use such an algorithm, but the features used in our experiments are based on the parameters C, S, H, V, P, Z (as described in section 2) that create six feature sets containing 21 values each, so all combinations of these sets can be considered. The total number of these combinations is 63, so the brute force algorithm can be used. The best combination obtained using cross-validation procedure was 63D feature set based on C, S and P parameters.

The next step was to find the best regularization method and corresponding parameter value. The method described by formula (2) and λ=0.8 gave the best results.

The RDA classifier uses a table of values of the discriminant functions $d_k(x)$ and assigns a test vector to a class with minimal value of this function. Using this algorithm the recognition ratio 55,6% was achieved.

It is easy to notice that the bigger value of the discriminant function for the class the smaller probability that the vector belongs to this class. Let us consider an SVM binary classifier that deals with i-th and j-th class. Assume that this classifier assigns the vector x to the class i. Then the difference with $d_i(x)$ and $d_{min}(x) = \min\{d_k(x)\}$, $k=1,2,\ldots, n$ can be treated as a measure of reliability of such a classifier. Formally this measure will be the weight of the vote and can be defined as:

$$1 - (d_i(x) - d_{min}(x)) / d_{min}(x) \quad (8)$$

Using this procedure to each binary classifier there is a weight assigned. These weights are applied to the voting table, so the votes are not equally treated. Now, the protein with the maximum number of votes (which is now a real number) is classified as the correct class. The recognition rate using this method is 61,8%.

Results and conclusions

In this paper there is presented a combined generative/discriminative classifier. This classifier uses the information provided

by the generative classifier, to improve the recognition rate of the discriminative classifier. The results using this method are presented in table 1. It can be seen that the combined classifier achieves better results than the SVM or RDA classifiers alone.

The accuracy measure used in this paper is the standard Q percentage accuracy (Baldi et al. [1]). Suppose there is $N=n_1+n_2+\ldots+n_p$ test proteins, where n_i is the number of proteins which belong to the class i. Suppose that c_i of proteins from n_i are correctly recognised (as belonging to the class i). So, the total number of $C=c_1+c_2+\ldots+c_p$ proteins is correctly recognized. Therefore the total accuracy is $Q= C/N$.

Table 2. Performance of the proposed classifiers

RDA	SVM	SVM-RDA
55,6%	58,7%	61,8%

The results achieved using the proposed strategies are promising. The recognition rates obtained using these algorithms (55,6%-61,8%) are comparable to those described in literature (48.8%-61.2%).

Table 3. Comparison between different methods

Method	Recognition ratio
SVM [5]	56,0%
HKNN [14]	57,4%
DIMLP-B [15]	61,2%
RS1_HKNN_K25 [13]	60,3%
RBFN [16]	51,2%
MLP [17]	48,8%
SVM-RDA (this paper)	61,8%

The obtained results are very encouraging. Our results improved the recognition ratio achieved by other methods proposed in literature, however, some extra experiments are needed, especially to consider other approaches to the multi-class SVM.

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KERAM: A NOVEL STAND-ALONE APPLICATION FOR CORRELATED MUTATIONS IDENTIFICATION AND ANALYSIS

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Abstract: Keram is a stand-alone Windows 2000/XP/Vista application designed for the detection and analysis of the correlated mutations. Study of this phenomenon provides important information about protein structure stability factors as well as the formation of protein complexes. It is generally assumed that the mechanism of compensation explains the mutations that occur simultaneously. Keram is designed to detect the mutational correlations by comparative analysis of multiple sequence alignments. Additionally a three dimensional structure can be applied to calculate the distance between correlated positions in the protein molecule.

Keram has been succesfully applied for the analysis of kinase subfamilies. The obtained data suggest that the mechanism of compensation does not explain utterly this phenomenon which seems to be much more complex and diverse. The residues that are detected as correlated are often placed at very distant positions of the protein structure, therefore the direct mutual interaction between them is impossible. We have detected not only correlated pairs, but also clusters of positions (even more than 10) that reveal correlated changeability.

Keywords: correlated mutations, multiple sequence alignment, protein-protein interaction

Background

Correlated mutations are the mutations occuring simultaneously at two or more positions and dependent on each other [Oliveira *et. al.,* 2002]. This phenomenon is presumed to be always concerned with the direct mutual interaction of the correlated positions [Neher, 1993]. It is assumed that correlated mutations give important information about protein-protein interaction [Valencia and Pazos, 2002] and/or by the compensation mechanism (Fig. 1). This theory involves local 'complementarity' of amino acids in a protein structure. The current approaches of protein folding prediction on the basis of known amino acid sequence are aided by the detection and analysis of correlated mutations.

So far the correlated mutations has been succesfully detected and analysed in the study of kinase families, serine proteinase families and protein inhibitor families with the aid of another application (CORM) developed by our group [Górecki *et al.*, 2005]. At present the CORM results are verified and confirmed by the Keram as an independent approach designed for the same purpose of theoretical protein analysis.

Implementation

At present the multiple sequence alignment in raw text format is accepted by Keram, fasta format is not supported. Keram searches for pairs that reveal mutational correlations.

Prior to analysis, the parameter of minimal variability must be declared for two positions suspected to be correlated. Variability is defined as the number of different amino acids that occupy the corresponding positions in the multiple sequence alignment. Obviously the minimal possible value is 2. There is a possibility to exclude about 5 upstream and downstream positions in primary structure to avoid trivial results for the pairs that interact directly e.g. in alpha helices. It is also possible to apply three more additional filters. The first one is the ratio, which enables to search for pairs that show correlation phenomenon. The second filter refers to the threshold value which is the minimal percentage of residues in analyzed positions that reveal correlation. The third filter that should be applied is the maximum variability difference value. This filter delimits the analysis to the positions in which number of different amino acids are mutually different. After setting all the required parameters, the analysis is executed.

Keram generates three types of output data. The first one and the most essential is the list of correlations (Fig. 3). The second output file is the correlation map (Fig. 4). This map is a graphic representation of the positions found to be correlated. The vertical and horizontal axes represent the sequence while the black dots mark the correlations. Keram prints also the data of distribution values of correlation for all 400 possible amino acid pairs (Fig. 5). These data contain average threshold and variability values for detected correlations. Keram is a user friendly application and does not require any advanced course to be applied.

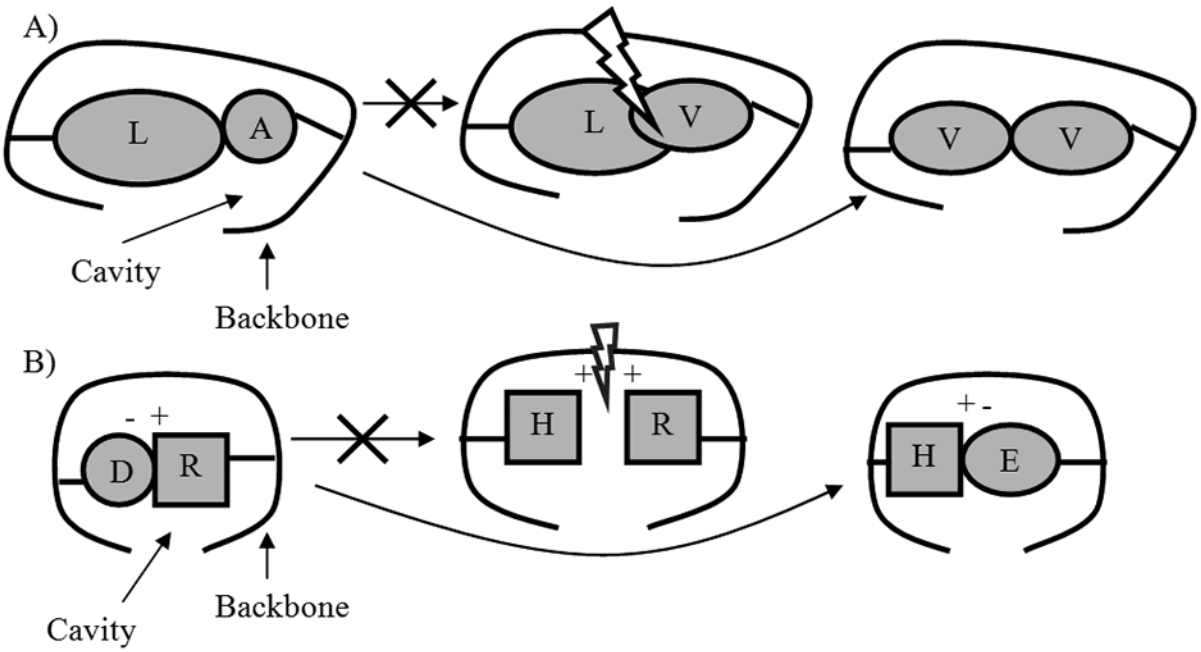

Fig. 1. A theoretical model showing the mechanism of compensation. Two residues that completely fill up a structural cavity must mutate simultaneously to preserve the structure. A) Two hydrophobic residues: leucine (large) and alanine (small) are simultaneously replaced by two valines (medium size). The single mutation of the alanine into valine is 'forbidden' due to size disruption. B) Two charged residues mutate simultaneously to maintain the charge arrangement. The single replacement of aspartic acid by histidine is not favored. Simultaneous mutation of aspartic acid into histidine and arginine into glutamic acid maintains the charge arrangement and does not disrupt the protein structure.

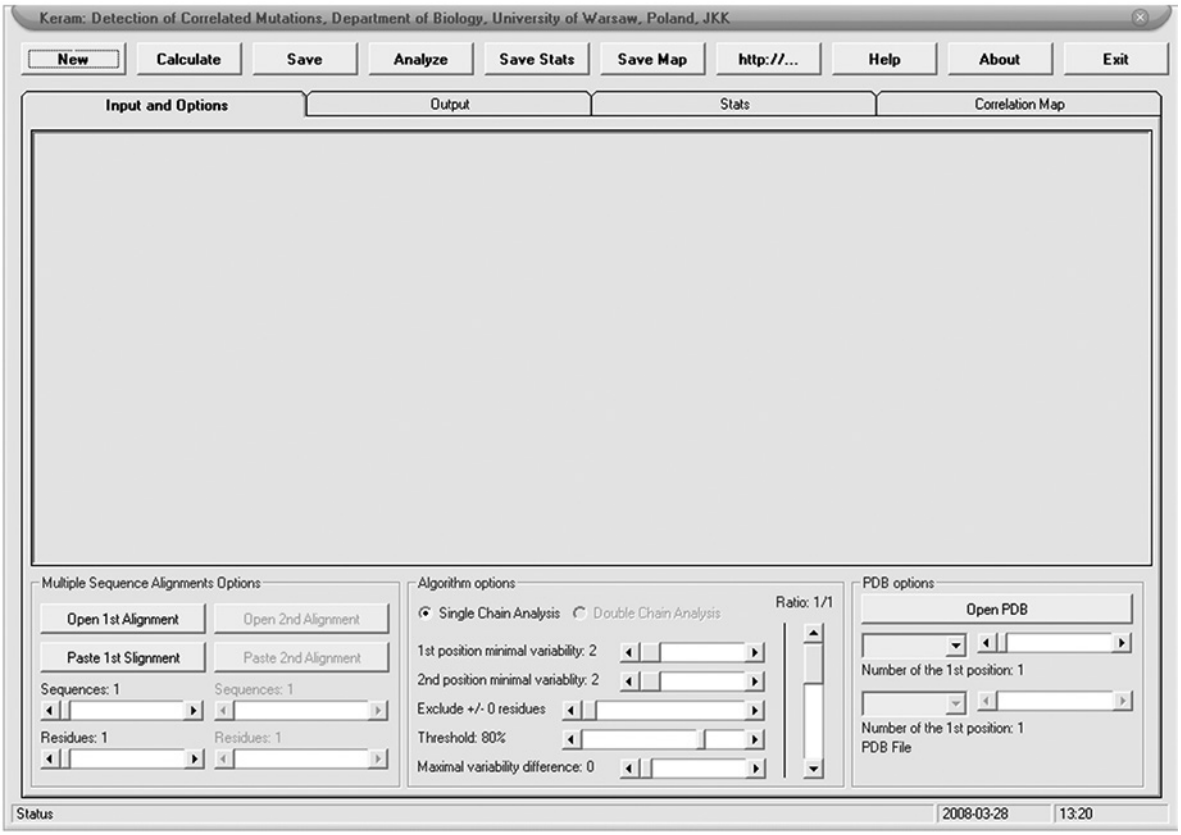

Fig. 2. Keram's GUI

```
Correlated Mutations detected by Keram
Start of the computation: 2009-06-13 12:01
Exclude +/- 0 residues, Threshold: 80%
Minimal variability of the 1st residue: 3
Minimal variability of the 2nd residue: 3
1     | 21   | 6  | L | 35   | 4  | G | 80%
2     | 21   | 6  | L | 36   | 5  | L | 82%
3     | 21   | 6  | L | 48   | 4  | M | 80%
4     | 21   | 6  | L | 57   | 3  | P | 82%
5     | 21   | 6  | L | 59   | 3  | G | 82%
6     | 21   | 6  | L | 61   | 3  | E | 82%
7     | 21   | 6  | L | 71   | 4  | G | 80%
8     | 21   | 6  | L | 72   | 3  | G | 82%
9     | 21   | 6  | L | 76   | 3  | R | 82%
10    | 21   | 6  | L | 122  | 6  | F | 80%
Part of this file was removed for brevity
2531  | 398  | 3  | D | 198  | 4  | T | 84%
2532  | 398  | 3  | D | 214  | 4  | I | 86%
2533  | 398  | 3  | D | 217  | 4  | T | 86%
2534  | 398  | 3  | D | 223  | 3  | Y | 84%
2535  | 398  | 3  | D | 274  | 6  | G | 82%
2536  | 398  | 3  | D | 276  | 4  | Q | 80%
2537  | 398  | 3  | D | 280  | 3  | K | 84%
2538  | 398  | 3  | D | 286  | 3  | Y | 82%
2539  | 398  | 3  | D | 366  | 3  | R | 86%
Computation end: 2009-06-13 12:35:58
```

Fig. 3. A typical list of correlated positions

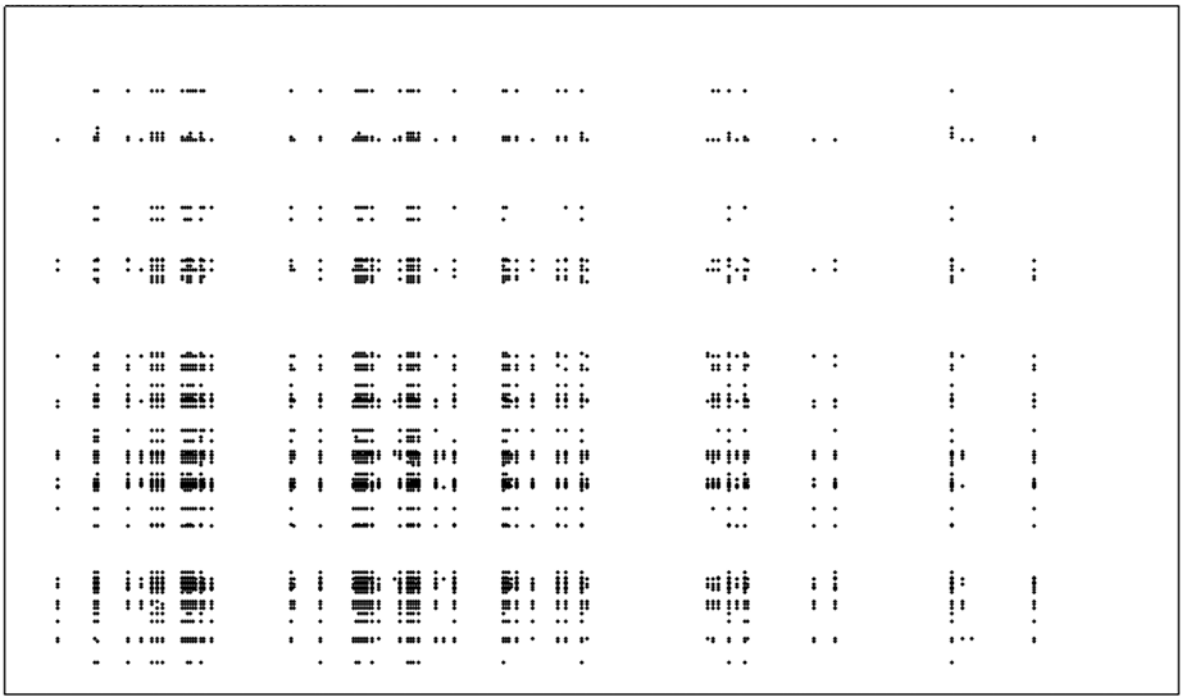

Fig. 4. Map of the correlated mutations. The vertical and the horizontal axis represent amino acid sequence of a protein. The dots represent the coordinates of the sequence positions that reveal simultaneous mutations

Results

Keram has been tested in the analysis of kinase families. The homologous amino acid sequences were obtained from the Uniprot [Apweiler *et al.,* 2004., Apweiler *et al.*, 2004., Bairoch *et al.*, 2005] and PFAM [Finn et al. 2006] databases. The multiple sequence alignment was achieved with the aid of ClustalX [Thompson *et al.*, 1994, Thompson *et al.*, 1997] and refined with the genetic semihomology algorithm [Leluk 1998, Leluk 2000ab, Leluk *et al.*, 2001, Leluk *et al.*, 2003]. For calculation of the distance distribution between correlated residues, the 3D structures were taken from the Protein Data Bank [Berman *et al.*, 2003] and ModBase [Pieper *et al.*, 2004, Pieper *et al.*, 2006]. The results showed serious inconsistency between the observed phenomenon of correlated mutations and its explanation by the theory of compensation. The correlated positions were often very dfistant from each other and direct or indirect mutual interaction was not possible. This suggests another mechanism of functional relationship between very distant positions that reveal mutational correlation. The correlation groups the related positions into clusters rather than pairs. The clusters may often consist of more than 10 positions.

Total amount of correlated pairs:

1952	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	0	3	4	6	0	1	3	17	0	0	14	3	2	11	6	3	11	0	1	5
R	0	1	4	4	0	2	2	16	0	0	14	2	2	11	4	2	10	0	1	10
N	0	3	3	4	0	1	3	17	0	0	11	3	1	13	6	2	11	0	1	7
D	0	3	6	4	0	2	3	17	0	0	14	3	3	13	6	3	14	0	2	8
C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Q	0	2	2	4	0	0	2	8	0	0	2	2	1	6	4	2	6	0	0	3
E	0	2	3	3	0	1	1	11	0	0	8	2	1	9	4	1	7	0	1	5
G	0	9	14	14	0	4	8	41	0	0	44	9	5	36	16	8	33	0	4	27
H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
I	0	1	2	2	0	0	1	5	0	0	5	1	1	5	2	1	5	0	1	3
L	0	10	12	11	0	1	10	51	0	0	35	10	7	37	19	6	33	0	3	22
K	0	2	4	4	0	2	2	14	0	0	14	1	2	11	4	2	10	0	2	10
M	0	1	1	2	0	1	1	5	0	0	6	1	0	4	2	1	5	0	1	2
F	0	6	10	9	0	3	6	32	0	0	30	6	4	20	11	5	21	0	3	18
P	0	2	4	4	0	2	2	14	0	0	14	2	2	11	2	2	10	0	2	9
S	0	2	3	2	0	1	2	11	0	0	6	2	1	7	4	0	7	0	1	5
T	0	7	12	14	0	2	7	40	0	0	37	7	6	29	14	7	27	0	6	24
W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y	0	3	4	6	0	2	3	15	0	0	16	3	3	12	6	3	15	0	2	8
V	0	6	6	6	0	1	4	26	0	0	19	6	2	18	9	4	18	0	1	10

Fig. 5. Correlated mutations distribution within all 400 possible amino acid pairs calculated by Keram

```
Percentage of correlated pairs:
100%|A   |R   |N   |D   |C   |Q   |E   |G   |H   |I   |L   |K   |M   |F   |P   |S   |T   |W   |Y   |V   |
A   |0   |0,15|0,20|0,30|0   |5,12|0,15|0,87|0   |0   |0,71|0,15|0,10|0,56|0,30|0,15|0,56|0   |5,12|0,25|
R   |0   |5,12|0,20|0,20|0   |0,10|0,10|0,81|0   |0   |0,71|0,10|0,10|0,56|0,20|0,10|0,51|0   |5,12|0,51|
N   |0   |0,15|0,15|0,20|0   |5,12|0,15|0,87|0   |0   |0,56|0,15|5,12|0,66|0,30|0,10|0,56|0   |5,12|0,35|
D   |0   |0,15|0,30|0,20|0   |0,10|0,15|0,87|0   |0   |0,71|0,15|0,15|0,66|0,30|0,15|0,71|0   |0,10|0,40|
C   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
Q   |0   |0,10|0,10|0,20|0   |0   |0,10|0,40|0   |0   |0,10|0,10|5,12|0,30|0,20|0,10|0,30|0   |0   |0,15|
E   |0   |0,10|0,15|0,15|0   |5,12|5,12|0,56|0   |0   |0,40|0,10|5,12|0,46|0,20|5,12|0,35|0   |5,12|0,25|
G   |0   |0,46|0,71|0,71|0   |0,20|0,40|2,10|0   |0   |2,25|0,46|0,25|1,84|0,81|0,40|1,69|0   |0,20|1,38|
H   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
I   |0   |5,12|0,10|0,10|0   |0   |5,12|0,25|0   |0   |0,25|5,12|5,12|0,25|0,10|5,12|0,25|0   |5,12|0,15|
L   |0   |0,51|0,61|0,56|0   |5,12|0,51|2,61|0   |0   |1,79|0,51|0,35|1,89|0,97|0,30|1,69|0   |0,15|1,12|
K   |0   |0,10|0,20|0,20|0   |0,10|0,10|0,71|0   |0   |0,71|5,12|0,10|0,56|0,20|0,10|0,51|0   |0,10|0,51|
M   |0   |5,12|5,12|0,10|0   |5,12|5,12|0,25|0   |0   |0,30|5,12|0   |0,20|0,10|5,12|0,25|0   |5,12|0,10|
F   |0   |0,30|0,51|0,46|0   |0,15|0,30|1,63|0   |0   |1,53|0,30|0,20|1,02|0,56|0,25|1,07|0   |0,15|0,92|
P   |0   |0,10|0,20|0,20|0   |0,10|0,10|0,71|0   |0   |0,71|0,10|0,10|0,56|0,10|0,10|0,51|0   |0,10|0,46|
S   |0   |0,10|0,15|0,10|0   |5,12|0,10|0,56|0   |0   |0,30|0,10|5,12|0,35|0,20|0   |0,35|0   |5,12|0,25|
T   |0   |0,35|0,61|0,71|0   |0,10|0,35|2,04|0   |0   |1,89|0,35|0,30|1,48|0,71|0,35|1,38|0   |0,30|1,22|
W   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
Y   |0   |0,15|0,20|0,30|0   |0,10|0,15|0,76|0   |0   |0,81|0,15|0,15|0,61|0,30|0,15|0,76|0   |0,10|0,40|
V   |0   |0,30|0,30|0,30|0   |5,12|0,20|1,33|0   |0   |0,97|0,30|0,10|0,92|0,46|0,20|0,92|0   |5,12|0,51|
```

Fig. 6. Percentage distribution of correlated mutations within all 400 possible amino acid pairs calculated by Keram

```
Average variability difference:
1,29|A   |R   |N   |D   |C   |Q   |E   |G   |H   |I   |L   |K   |M   |F   |P   |S   |T   |W   |Y   |V   |
A   |0   |1,33|1,5 |1   |0   |1   |1,33|0,88|0   |0   |0,57|1,33|0,5 |0,54|0,83|0,66|0,54|0   |1   |0,4 |
R   |0   |0   |2   |1   |0   |3   |0   |1   |0   |0   |2   |0   |1   |1,81|0,5 |2   |1,2 |0   |1   |2,6 |
N   |0   |1,33|2,66|2   |0   |3   |1,33|1,58|0   |0   |2,18|1,33|1   |1,84|1,5 |2   |1,81|0   |1   |2,28|
D   |0   |0,66|2   |1,5 |0   |2   |0,66|1   |0   |0   |1,35|0,66|1   |1,46|0,83|1,33|1,07|0   |1   |1,37|
C   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
Q   |0   |2   |2   |1,5 |0   |0   |2   |1,75|0   |0   |0,5 |2   |2   |1   |1,5 |1   |0,66|0   |0   |1   |
E   |0   |0,5 |1,66|1   |0   |3   |1   |0,90|0   |0   |1,87|0,5 |1   |1,22|0,5 |2   |1   |0   |1   |2   |
G   |0   |1,22|2,07|1,35|0   |2,75|1,25|1,48|0   |0   |1,52|1,22|0,6 |1,36|1,12|1,25|1   |0   |0,75|1,96|
H   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
I   |0   |1   |2   |1   |0   |0   |1   |0,6 |0   |0   |0,8 |1   |0   |0,8 |0,5 |1   |0,2 |0   |0   |1   |
L   |0   |2,2 |2,16|1,09|0   |1   |2,2 |1,68|0   |0   |1   |2,2 |1   |1,18|1,73|0,83|1   |0   |1   |1,31|
K   |0   |0   |2   |1   |0   |3   |0   |0,92|0   |0   |2   |0   |1   |1,81|0,5 |2   |1,2 |0   |1   |2,6 |
M   |0   |1   |1   |1   |0   |2   |1   |0,6 |0   |0   |1   |1   |0   |0,5 |0,5 |1   |0,2 |0   |0   |1   |
F   |0   |1,83|2,1 |1,44|0   |2   |1,83|1,34|0   |0   |0,96|1,83|0,5 |1,3 |1,36|1   |0,66|0   |0   |1,5 |
P   |0   |0,5 |2   |1   |0   |2,5 |0,5 |0,92|0   |0   |1,5 |0,5 |0,5 |1,36|1   |1,5 |0,7 |0   |0,5 |1,88|
S   |0   |1,5 |1,66|1   |0   |1   |1,5 |1,09|0   |0   |0,83|1,5 |1   |0,71|1   |0   |0,71|0   |1   |0,8 |
T   |0   |1,42|2   |1,14|0   |1,5 |1,42|1,07|0   |0   |0,81|1,42|0,16|0,75|0,92|0,85|0,44|0   |0,16|1,16|
W   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
Y   |0   |0,33|1,25|1   |0   |3   |0,33|0,66|0   |0   |1,81|0,33|0,66|1,25|0,5 |1,66|0,86|0   |1   |2   |
V   |0   |2,66|2,5 |1,33|0   |2   |2,5 |1,96|0   |0   |1,15|2,66|1   |1,5 |1,88|1   |1,22|0   |0   |2   |
```

Fig. 7. Average variability variety values for correlated pairs calculated by Keram

```
Average Threshold:
85,4|A   |R   |N   |D   |C   |Q   |E   |G   |H   |I   |L   |K   |M   |F   |P   |S   |T   |W   |Y   |V   |
A   |0   |87,3|84  |83,6|0   |82  |86,6|84,8|0   |0   |82,5|87,3|83  |84,3|86,3|84  |83,4|0   |82  |84  |
R   |0   |94  |88  |90  |0   |82  |94  |88,2|0   |0   |87  |95  |88  |88,5|93,5|91  |88,6|0   |84  |84,4|
N   |0   |86,6|82  |86  |0   |80  |85,3|84,2|0   |0   |83,8|86,6|86  |83,6|85  |85  |84,5|0   |84  |83,7|
D   |0   |90  |85,3|87  |0   |80  |88  |87,0|0   |0   |84,1|90  |83,3|85,3|88,6|87,3|85,5|0   |83  |84  |
C   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
Q   |0   |83  |82  |82  |0   |0   |83  |83,2|0   |0   |82  |83  |80  |81  |83  |82  |81,3|0   |0   |80,6|
E   |0   |88  |85,3|86  |0   |84  |82  |86,1|0   |0   |86  |88  |90  |85,3|87,5|92  |86,2|0   |84  |84,4|
G   |0   |88  |85  |86,7|0   |83  |88,5|86  |0   |0   |84,8|88  |87,2|85,9|88,1|86,2|86,2|0   |84,5|83,7|
H   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
I   |0   |90  |86  |88  |0   |0   |88  |87,6|0   |0   |83,2|90  |82  |84,4|88  |86  |85,2|0   |82  |83,3|
L   |0   |85,2|83,6|84,7|0   |80  |85  |84,3|0   |0   |83,4|85,2|82,5|84,3|84,8|85,3|84,1|0   |80  |83  |
K   |0   |94  |88  |89,5|0   |81  |93  |88,8|0   |0   |86,5|92  |87  |88,1|92,5|90  |89  |0   |84  |84,4|
M   |0   |88  |86  |85  |0   |80  |90  |87,2|0   |0   |83  |88  |0   |85,5|89  |88  |84  |0   |82  |83  |
F   |0   |88,3|84,6|86,2|0   |80,6|87,6|86,5|0   |0   |85,2|88,3|85,5|85,4|88  |86,8|86,9|0   |82  |84  |
P   |0   |94  |87  |90  |0   |83  |94  |89  |0   |0   |86,2|94  |89  |88  |92  |92  |88,4|0   |84  |84,4|
S   |0   |86  |83,3|89  |0   |82  |86  |84,7|0   |0   |85,3|86  |88  |85,1|86  |0   |84,8|0   |82  |82,8|
T   |0   |88,5|85  |86  |0   |81  |87,4|86,2|0   |0   |84,4|88,5|84  |86,0|87,7|86,2|85,1|0   |81,3|83  |
W   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |0   |
Y   |0   |88,6|87  |86,3|0   |80  |88,6|87,7|0   |0   |84,3|88,6|85,3|86,3|88,3|86  |85,3|0   |84  |83,7|
V   |0   |84,3|84  |84,3|0   |80  |85  |83,8|0   |0   |83,4|84,3|83  |84  |84,4|83,5|83,4|0   |82  |82  |
```

Fig. 8. Average threshold values calculated for each possible pair of amino acids

Conclusions

The Keram application has been successfully applied in the analysis of correlated mutations. It's intuitive interface easily detects and shows the positions that reveal simultaneous mutations. The obtained results indicate that simultaneous mutations are not limited to contacting amino acid pairs. It is suggested that there exist long distance clustering relationships that manifest correlated mutational variability.

Comparing to other similar applications [Kass and Horovitz, 2002] Keram can be used offline as a stand-alone software installed on individual PC. The Keram's output files can be expolited to carry more advanced analysis with the aid of other software offered by our group.

The results achieved by Keram are consistent with the results obtained with the aid of CORM [Górecki *et al.*, 2005]. These two applications corroborate each other's results by independent confirmation of the accuracy and reliability. Additionally Keram supports the results of CORM by the graphic visualization of the detected correlated mutations.

Keram is freely accessible for the non-commercial use at: http://www.republika.pl/bioware. Also, the source code is available upon direct request to the authors.

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TEST-SIGNAL GENERATORS FOR MINDSET MS-1000 ELECTROENCEPHALOGRAPH WITH DATA ACQUISITION SYSTEM FOR LINUX OS

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Abstract: The paper presents a hardware and software solution giving methods of communication and the construction of the artificial EEG patients. The software is designed for the Mindset MS-1000 EEG 16-channel device, manufactured by Nolan Computer Systems. The modular analysing system for Linux OS is presented. In addition, to testify the calibration of the device – the artificial patient has been designed and constructed together with appropriate hardware-software generators.

Introduction

In the Division of Complex Systems and Neurodynamics of Computer Science Institute of Maria Curie-Skłodowska University a software for data acquisition and processing is being developed [1]. The software is designed for the Mindset MS-1000 EEG device, manufactured by Nolan Computer Systems. This device contains a 16-channel analog amplifier, a 16-bit analog-to-digital converter and a gathered data transmission trace.

Fig. 1. Example of EEG examination set

The device control and data interchange is operated by a SCSI interface [2][3]. An appropriate SCSI commands set makes it possible to control the sampling rate value (64, 128, 256, 512 or 1024 samples per second) and to begin and finish the process of data sampling [4].

The goal and general idea

The goal is to develop (under Linux OS) a complete examination system for the versatile analysis and visualization of EEG signals.

The idea of the project is to build a modular system, where individual modules are as much independent as possible, with a wide choice of active modules and an ordering option. A pipeline was chosen as an inter-process communication background.

A calibrator is an optional equipment for the EEG Mindset MS-1000 device. It is a sinusoidal, 16 Hz, 50 μV signal oscillator. Signal parameters of this oscillator are good. The deviations are: frequency below 1%, magnitude below 2% and harmonics factor below 1%. The main purpose of this calibrator is to determine scaling factors for data errors corrections – due to factors such as electronic components tolerance, small errors may occur in the process of data gathering [5].

Realisation: EEG test signal oscillators

At the first stage, a daemon program was developed. The role of the daemon is to control the Mindset MS-1000 EEG device and to receive data from it. An oscilloscope program was also developed to show the gathered data on the screen [6]. At this stage the calibrator was used as a signal oscillator.

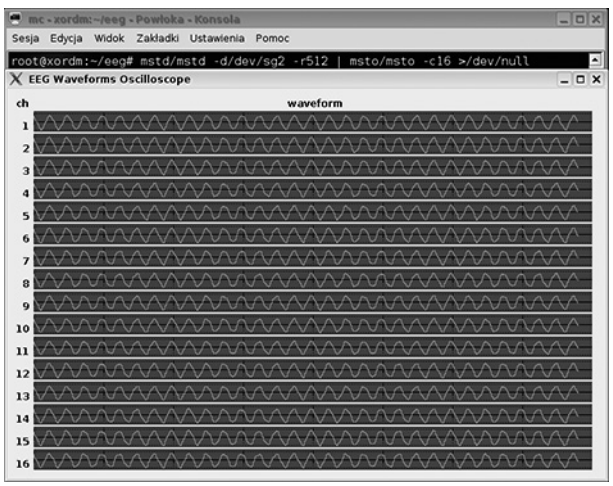

Fig. 2. Two seconds of calibrator signal (512sps)

One of the next stages of the system development will be the frequency spectrum analysis of the signal with Fourier transform [7]. For a more reliable testing of this system module, a more advanced signal oscillator is necessary.

It is possible to buy signal oscillators for the testing of the EEG equipment (sometimes called *artificial patient*), however, they usually contain only one function oscillator that generates a single sinusoidal, rectangular or triangular signal with variable frequency.

Rectangular and triangular waves are rich in odds harmonics, and this feature makes the testing of frequency spectrum analysis possible. We exactly know what the expected result is, because the waveforms are described in the form of the following formulas:

– for a symmetric rectangular waveform [8]:

$$x(t) = \frac{4}{\Pi}\left[\sin(\omega t) + \frac{1}{3}\sin(\omega 3t) + \frac{1}{5}\sin(\omega 5t) + \frac{1}{7}\sin(\omega 7t) + \frac{1}{9}\sin(\omega 9t) + \ldots\right]$$

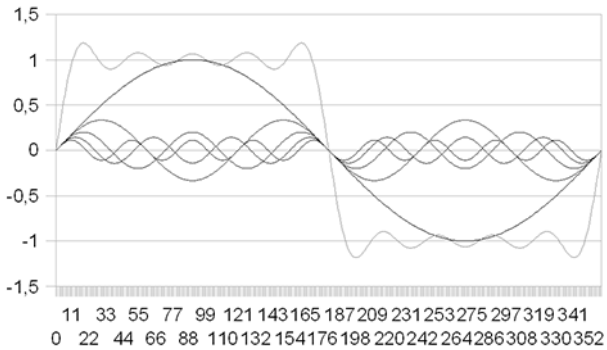

Fig. 3. First five components and formula resultant (1)

– for a symmetric triangular waveform [8]:

$$x(t) = \frac{8}{\Pi^2}\left[\sin(\omega t) + \frac{1}{3^2}\sin(\omega 3t) + \frac{1}{5^2}\sin(\omega 5t) + \frac{1}{7^2}\sin(\omega 7t) + \frac{1}{9^2}\sin(\omega 9t) + \ldots\right]$$

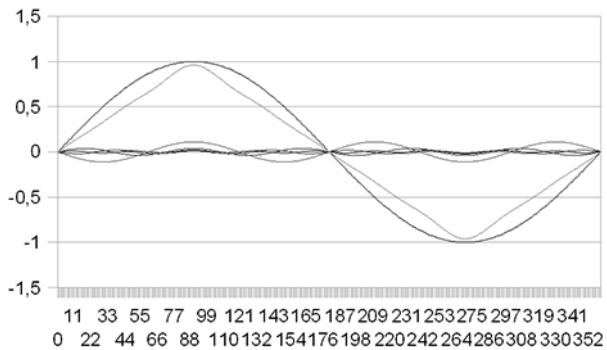

Fig. 4. First five components and formula resultant (2)

As the above formulas show, the content of higher harmonics is much lower for the triangular waveform than for the rectangular one. This is expressed by the harmonics factor, the value of which is about 12% for the triangular and 45% for the rectangular waveform [9].

In order to have a more versatile possibility of shaping the test signal, an oscillator containing two independent function oscillators was built. Additionally, signals from the function oscillators can be mixed together and analoguely multiplied (amplitude modulation).

The described oscillator is shown here: (Fig. 5).

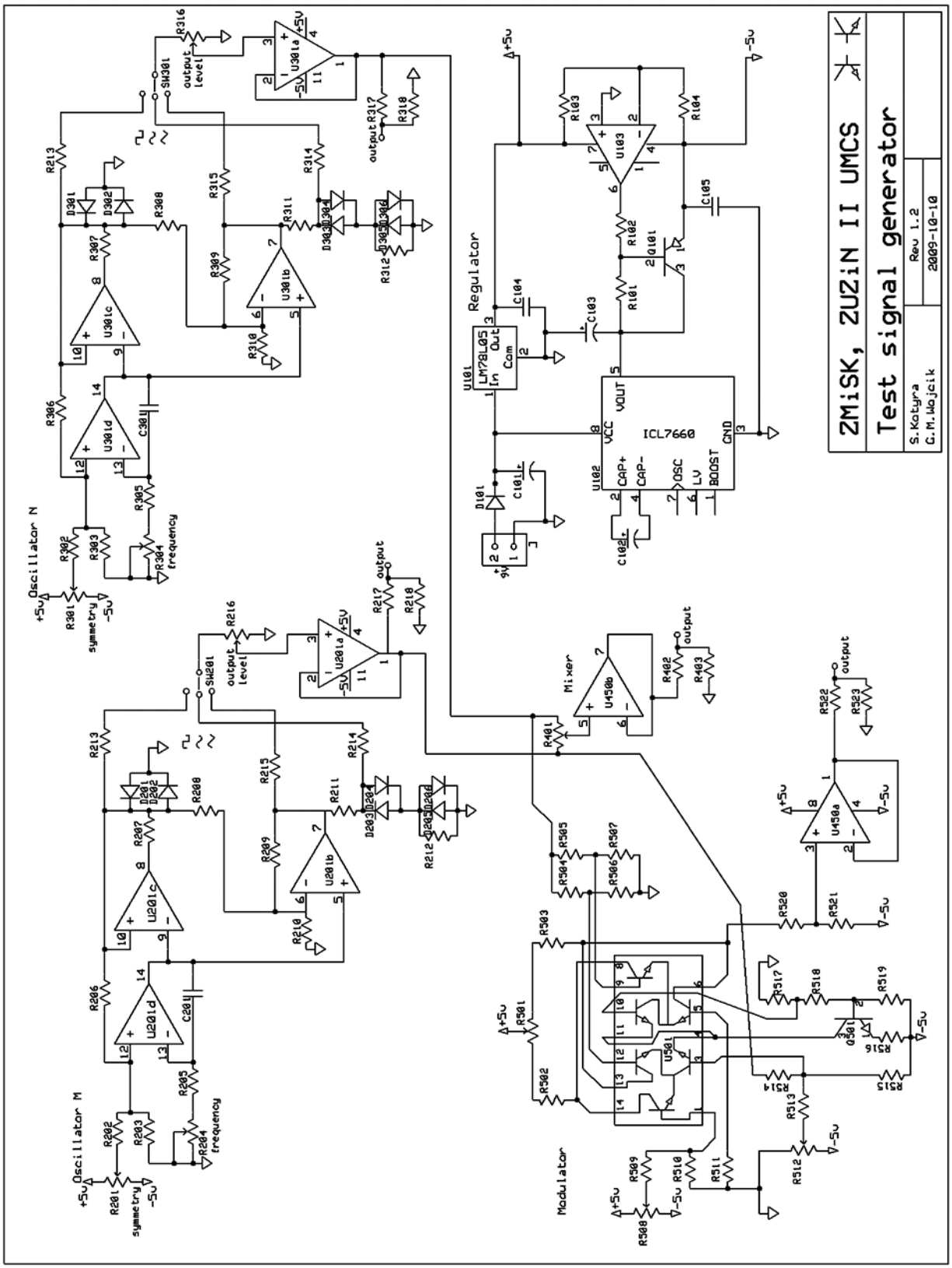

Fig. 5. Oscillator schema

A regulator (U101, U102) converts a single +9V voltage source to symmetric +5V, -5V voltages, which are used to power the whole circuit [10][11].

The function oscillator is based on a quad operational amplifier U201 (U301).

An integrator (U201d (U301d)) generates a triangular waveform. In order to do this, it has to cooperate with a threshold flip-flop (U201c (U301c)), on which a rectangular waveform is generated [12] (the voltage spike is eliminated on U201b (U301b)).

The shaping circuit converts the triangular waveform to a sinusoidal one using diodes D203..D206 (D302..D306) [13].

Switch SW201 (SW301) makes it possible to select the desired type of the waveform.

Potentiometer R204 (R304) changes the frequency of the generated signal, and potentiometer R216 (R316) changes its output voltage level.

Potentiometer R401 mixes the signals. The amplitude modulation (ring modulator) is based on U501 integrated circuit and Q501 transistor (it is an analogue signal multiplifier) [14].

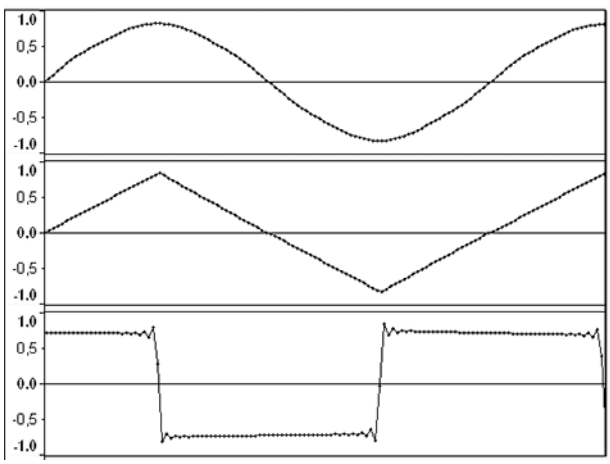

Fig. 6. Generated waveforms

The harmonics factor of a sinusoidal waveform doesn't exceed 2,5% (Fig. 7):

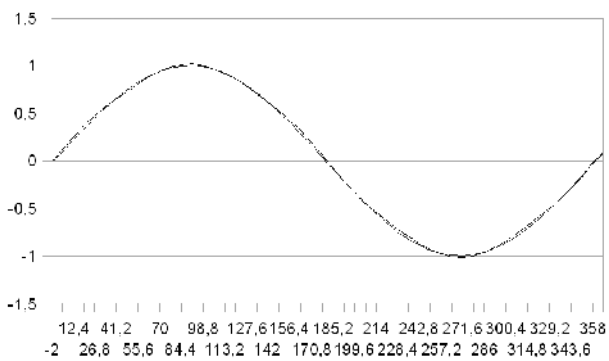

Fig. 7. Sinusoidal waveform distortion caused by harmonics

In the mixer we can get a signal described by following formula: $U_m = xU_M + (1-x)U_N$ where $0 \leq x \leq 1$

A solution like this guarantees that the EEG device will not be over-driven.

A very small (circa 10 ohms) output impedance of circuits (oscillators, mixer, modulator) makes the signal trace less prone to noise and interferences. This makes the testing conditions of the whole research better.

Fig. 8. EEG test signals generator

Shown on the back wall (Fig. 8) DB25 connector enables the use of an electro-cap cable.

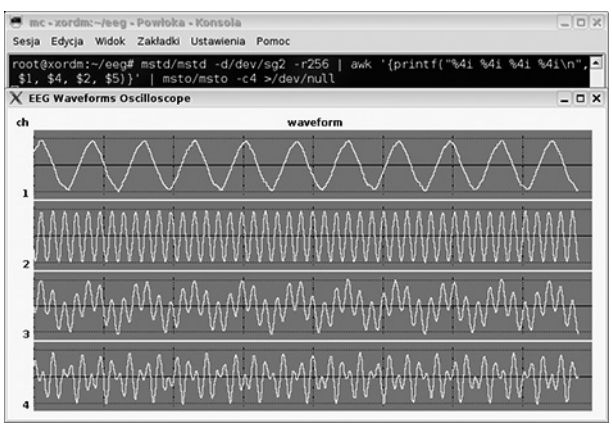

Fig. 9. Oscillogram of generated signals after passing through signal trace (256sps)

Fig. 9: channel 1 – sinusoidal waveform 2,8Hz, channel 2 – sinusoidal waveform 12Hz, channel 3 – signals from channel 1 and 2 mixed with 50% + 50% ratio, channel 4 – analog product (multiplying) of signals 1 and 2.

The daemon is a source of data stream corresponding to the measured data coming from the Mindset MS-1000 EEG device. It is possible to replace the daemon with a software generator for testing purposes.

A solution like this makes it possible to develop and test modules without real access to hardware such as the interface card, the EEG device and the signal generator.

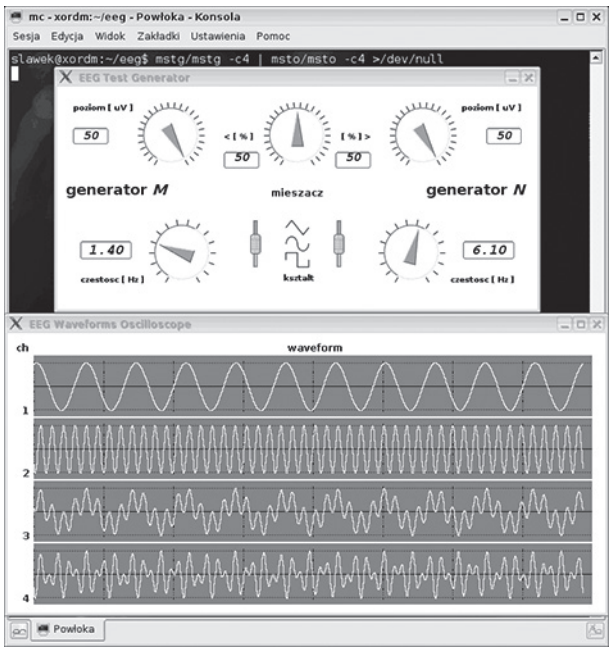

Fig. 10. Front panel of software generator and examples of waveforms (128sps)

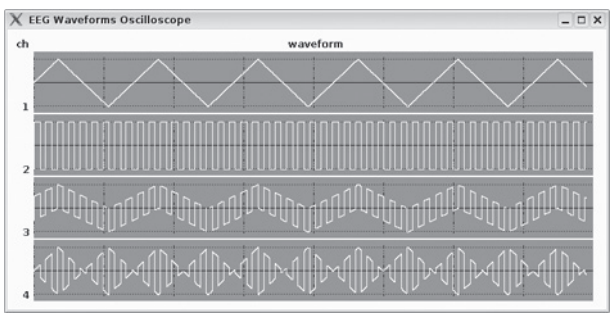

Fig. 11. Another example of software generator waveforms

Conclusions

In this paper EEG signal generators were presented. The software generator was designed to work under control of Linux OS. For this study we used Ubuntu 9.10, however, the philosophy of SCSI interface allows us to run the software in each distribution [15] [16]. This signal generator, which is in fact the artificial patient, is useful for optimal developing of other modules without the necessity of having access to the real Mindset. At this stage we have a fully free and functional EEG system working under control of Linux OS. Knowledge of the functionalities of the whole system will let us construct new, unsupported modules for EEG treatment methods and analysis.

Acknowledgments

The necessary information about the device and computer co-operation protocol through SCSI interface was kindly provided by Mr Wayne Nolan from Nolan Computer Systems, Colorado. The authors would like to thank Mr Nolan for his invaluable help.

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SIGNAL VISUALISATION SOFTWARE FOR MINDSET MS-1000 ELECTROENCEPHALOGRAPH

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Abstract: The paper presents EEG signal visualisation software designed for the Mindset MS-1000 EEG 16-channel device, manufactured by Nolan Computer Systems. The software is designed to work system independently and be accessible via web browsers. The presented application was developed in Adobe Flex technology with external Papervision3D library. It provides complete visualisation solutions such as navigable EEG chart or two separated 3D head models with applied EEG data.

Introduction

In the Division of Complex Systems and Neurodynamics of Computer Science Institute of Maria Curie-Sklodowska University a software for EEG data visualisation is being developed. The software is designed for the Mindset MS-1000 EEG device, manufactured by Nolan Computer Systems [1].

This device contains a 16-channel analog amplifier, a 16-bit analogue-to-digital converter and a gathered data transmission trace. The device control and data interchange is operated by a SCSI interface. An appropriate SCSI commands set makes it possible to control the sampling rate value (64, 128, 256, 512 or 1024 samples per second) and to begin and finish the process of data sampling [2-3]. According to the Nyquist frequency it is far enough to record human brain electrical activity that in natural conditions generates signal at frequency rate value from 1Hz to 100Hz.

Fig. 1. Mindset 1000 EEG System main device

The goal and general idea

Our goal was to develop EEG visualisation project for Mindset MS-1000 device. The idea is to create usable, commonly available and environment independent application. To provide best usability, EEG signal is visualised on a 3D head model that allows interaction with user's control device. Taking into account the high significance of the Internet nowadays and diversification of users operating systems, the main requirement for application is to work in a web browser as well as desktop and to be completely independent of user's operating system. Besides that, to ensure flexibility of development and decrease its costs the application is created only with tools that are open source and available free of charge.

Methods

The application was created in Adobe Flex [4-6] technology with external library Papervision3D [7-8].

Flex technology is used for development of Rich Internet Applications (RIA) which is the type of application that runs in a web browser and has capabilities comparable to desktop applications. One of the greatest RIA's functionalities is executing application on the client's side, what is significant for keeping high performance of servers and decreasing Internet connections traffic. As a consequence, once application is loaded it does not require to be refreshed after each user's action, what is typical for HTML based web applications [9].

The cross-platform character of Flex applications is acquired by Flash Player, which is a virtual machine for all Flash technology

based applications. Flex applications are compiled to swf format files, which are afterwards executed by proper Flash Player version installed for a specific user's operating system.

Flex sources are included with Flex SKD, which is provided free of charge. As a programming environment an education version of Flex Builder was chosen.

In order to display head model with applied EEG visualisation in three dimensions the Papervision3D library was used. Papervision3D is one of the most advanced and commonly advised 3D libraries for Flash technology. It is capable of rendering 3D scene in a real time and it supports external 3D models saved in various popular formats [10].

As a 3D modelling tool needed to create 3D head model the Blender was chosen. It is a complex and up-to-date well documented tool with a strong support from Internet society as well as from publishers [11].

Application

The main application class BEAV (Brain Electrical Activity Visualisation), apart from initialising all other major classes, is responsible for reading file with real EGG samples and putting them into array, which is the basic on which application components operate [12].

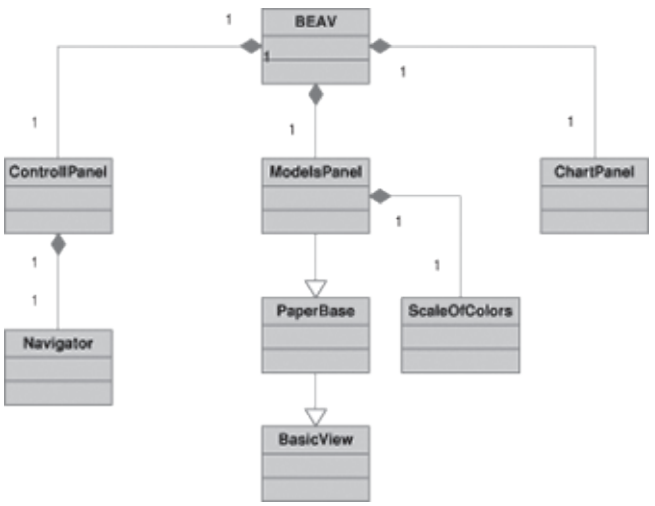

Fig. 2. UML diagram of the application

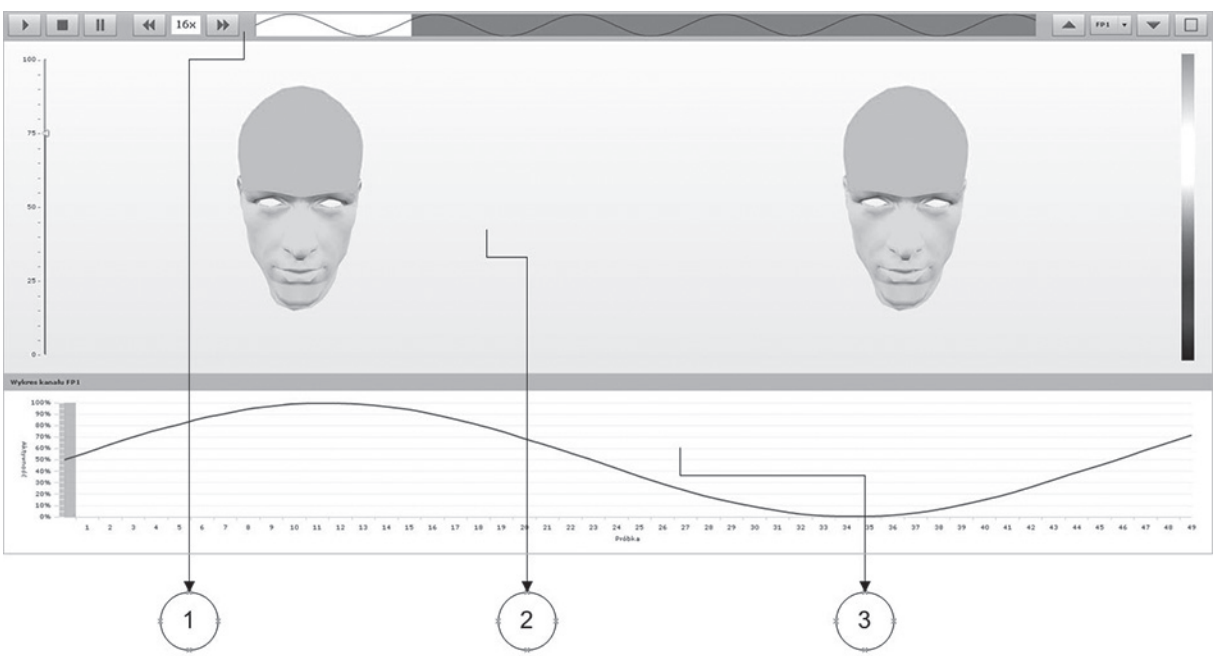

Fig. 3. Organisation of the application graphical interface: control panel (1), models panel (2), chart panel (3)

The application is organised into three panels: control panel, models panel, chart panel .

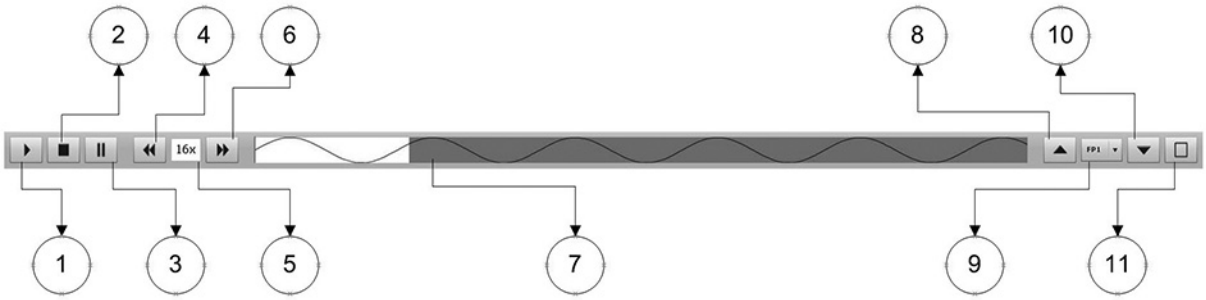

Fig. 4. Control Panel: play button (1), stop button (2), pause button (3), decrease samples per second button (4), samples per second label (5), increase samples per second button (6), navigator component (7), previous EEG channel button (8), EEG channels combo box (9), next EEG channel button (10), application screen mode button (11)

Control panel is used to play, stop and pause EEG signal, change samples per second playing rate, change electrode whose signal is drawn on the chart panel and switch between full-screen and normal screen mode. Besides that, it contains Navigator, which is the component created to navigate the signal drawn on the chart panel.

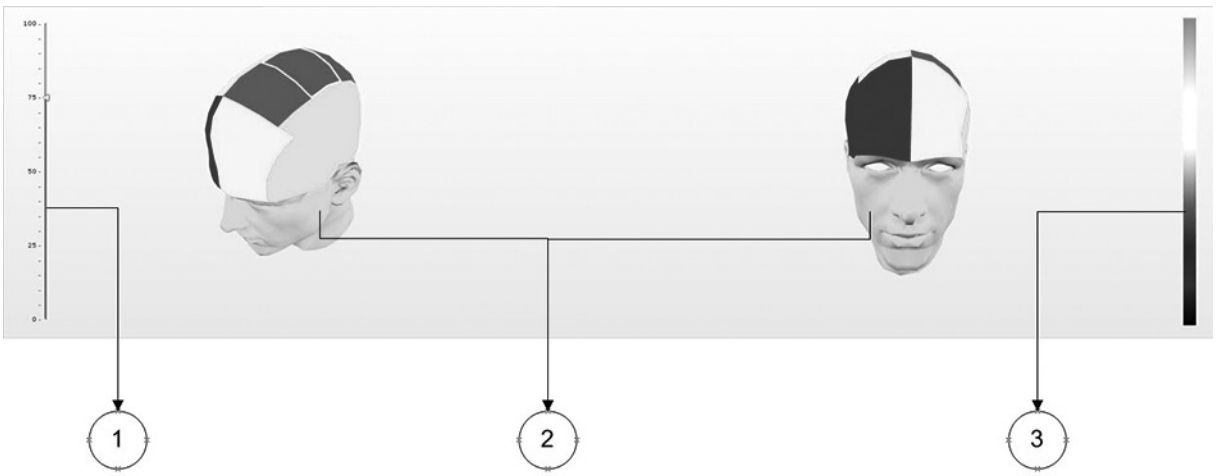

Fig. 5. Models panel: models' scale slider (1), head models (2), scale of colours (3)

ModelsPanel class inherits from PaperBase class, which is an extension of BasicView class. BasicView comes from Papervision3D library and contains functions and fields essential to set up a basic 3D scene.

To increase usability of the application there are displayed two head models. Therefore it was necessary to extend BasicView with custom PaperBase class. PaperBase adds additional camera and viewport to BasicView and it contains methods that handle all interactions with 3D models. Displayed head models are the same instance of single model added the stage but rendered twice on two viewports and being seen by two cameras that can be set at different position in the 3D space.

In order to visualise electrical activity on the model's scalp, proper areas (triangles) were selected with Blender from the 3D head model and marked as separate objects. Thereby it was possible to apply different colour materials to each area described by 10-20 international system of electrodes placement.

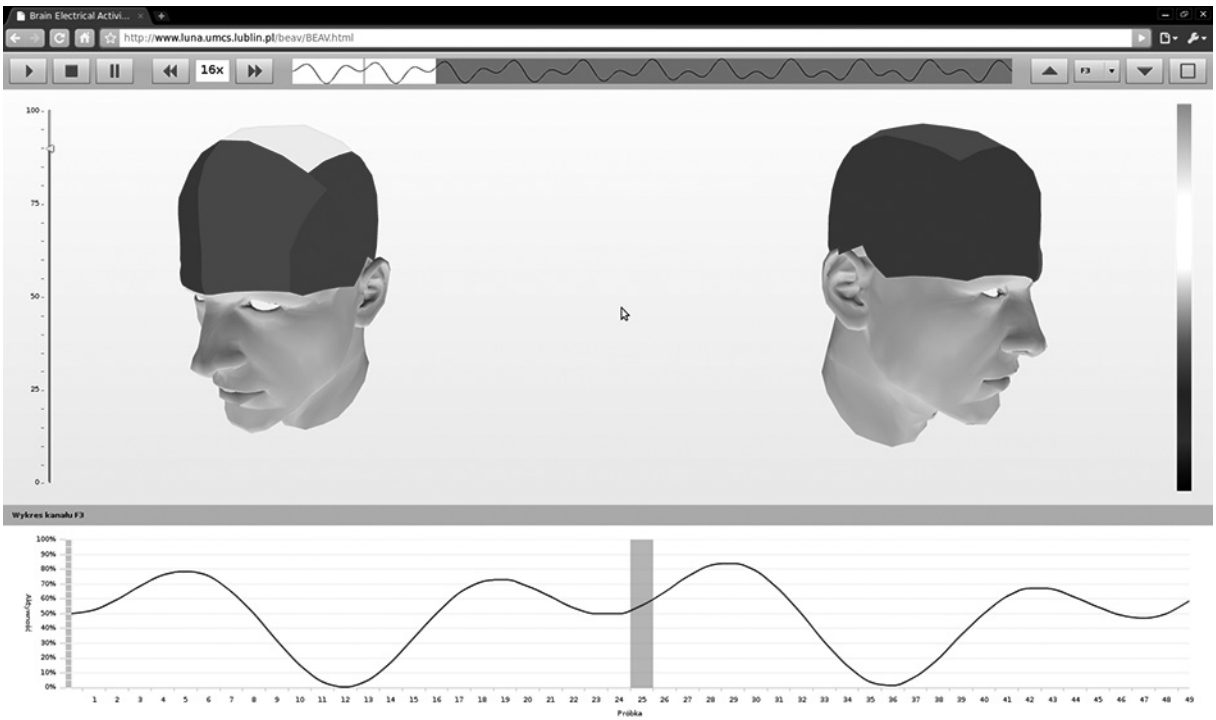

Fig. 6. Usage of the visualisation application in a web browser

Brain electrical activity saved in a file is casted on a scale of colours from dark blue to red, which shows properly areas of no electrical activity and highest electrical activity of brain. To make it easier, while looking at a given colour, to decide how active the chosen area is, the scale of colours bar was added to the models panel.

In order to adjust models size to screen resolution scale, a slider was added to models panel.

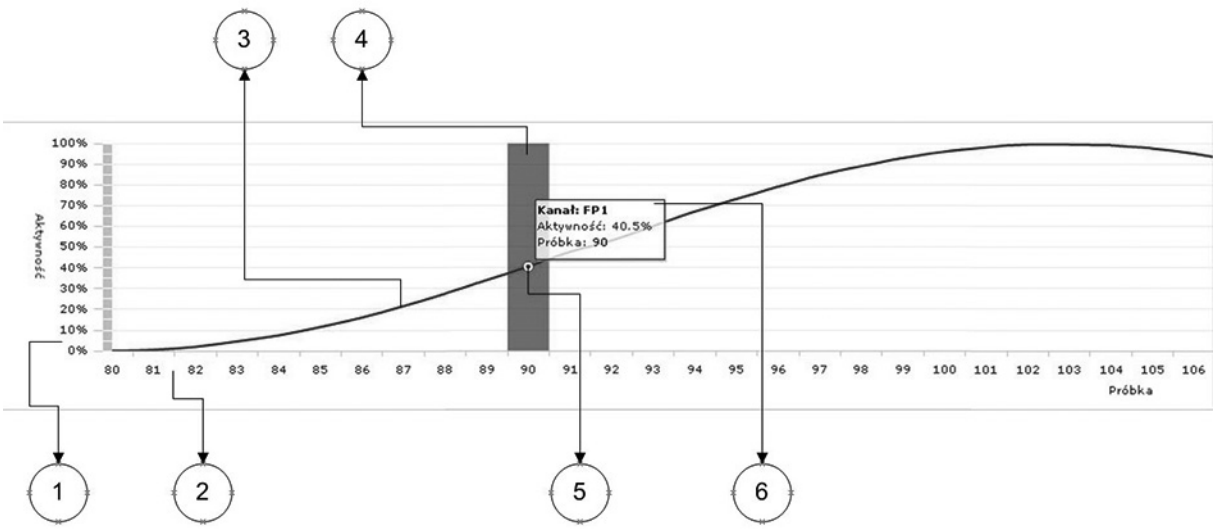

Fig. 7. Chart panel: brain activity axis (1), samples axis (2), EEG channel's chart (3), currently played sample's highlight (4), currently played sample (5), currently played sample's tool-tip box (6)

Chart panel contains LineChart object from the Flex SKD, which is used to draw a part of a EEG signal data. A piece of the signal drawn in chart panel is described by the navigator component from control panel. Chart panel provides red colour highlight of the currently visualised sample and tool-tip that contains information about sample e.g. activity represented by samples, it's number or name of the electrode (channel) that it belongs to.

The application, its current and future functionalities can be tested by everyone at [12]. Real EEG data sets are available for visualisation.

Summary

In this paper EEG signal visualisation software designed for the Mindset MS-1000 EEG device was presented. At this stage we have a fully free and functional EEG visualisation software, working under all popular operating systems and web browsers. The developed application can be considered as the base for further improvements like EEG signal analysis or live visualisation feature.

At the moment the application is in its initial stage. Being the part of one of the authors' PhD project, new functionalities are going to be added. Visualisation of many EEG tracks at the same time is considered, as well as adding possibility of visualising the activity not only on the head but also on the brain map. It will also be possible to add some markers that will allow to bookmark interesting moments in time series. The main development stages will be reported in next issues of BAMS.

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