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Intracranial Liquid Volume Fluctuations: Phenomenology and Physiological Background

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Abstract

The main features of periodical volume fluctuations of liquid media (blood and CSF) inside the crania-spinal cavity are the wide ranges of frequencies and amplitude variations; which involve the entire cavity and all its segments. These fluctuations vary from fast fluctuations - pulse and respiratory - too much slower fluctuations. This represents the combined result and interaction of a large number of physical forces initiated by many different physiological processes. It is possible to separate out three main processes responsible for crania-spinal liquid volume fluctuations. The first process is fluctuations in the central hemodynamics, the second process– fluctuations in local activity in the brain structures and related changes of the brain vascular tone. These are connected with the brain's cellular metabolism. In the first two process groups the overriding executive mechanism for liquid volume fluctuation is the cerebrovascular system. However, all these processes are acting simultaneously. The final indices of intracranial liquids fluctuations are very complicated with changeable frequency ranges of 0.1–0.3 Hz and with amplitudes of 0.2–0.8 compared with pulse changes. The integrated nature of these fluctuations, which have been referred to as "slow fluctuations", indicate that slow volume fluctuations of intracranial origin could provide significant and valuable information concerning brain function and their relevance to the supporting circulatory and metabolic mechanisms. These slow fluctuations can be recorded by a number of methodologies; in particular "Rheoencephalography" (REG). REG is based on the measurement of electrical impedance between plate electrodes placed on human head. However, this measurement escaped the attention of many investigators due to the difficulties in quantitative analysis. Recently, due to advanced computerization, it was possible to provide definitive and accurate analyses. The aim of this paper is to show some peculiarities in the slow frequencies in intracranial liquid fluctuations and the correlations of these changes to quantitative indices under different physiological situations.

Keywords: Low frequency volume fluctuations; Rheoencephalography; Pulse amplitude; Normalized spectrum analysis

Introduction

At the end of the eighteenth century it was demonstrated that both intracranial cerebrospinal fluid (CSF) and cerebrovascular blood circulation (CBS) could be functionally characterized as periodically fluctuating systems not connected to either heart or respiratory activities [1,2]. However, some decades later the question was arose whether these volume fluctuations are inherent only to the cranium as they appear in open skull situations. The reason for this question was the use of new technology and observations on animal brain surfaces (with cats and dogs) through hermetical transparent skull "windows". These studies indicated the absence of heart and respiratory pulsations but other independent volume fluctuations inside a closed cranium [3,4]. At the same time, some investigators, based on their experimental data received through data collected by other instrumentation, supported the concept of the presence of slow liquid volume fluctuations inside an intact craniospinal cavity [5-10].

Oxygen fluctuations

The next step in the study of slow fluctuations inside the craniospinal cavity was made possible by developing special experimental techniques based on advances in electronic hardware over 50-60 years. This period could be regarded as the start of systematic investigations into slow intracranial fluctuations. For this purpose direct high frequency alternating currents were used. Based on direct current polarography the methods for recording of oxygen and hydrogen availability (pO2 and H2) in brain tissue were constructed. Recordings of pO2 are based on inserting insulated fine gold or platinum wire electrode with small working surfaces directly into brain tissue. The second referent electrode with a comparatively larger surface was then placed on the head's skin surface. Both electrodes were connected with a direct voltage source (0.3-0.8 V). The sending electrode was connected with a minus charge and the referent electrode with a positive charge. The value of current in this circuit will be proportional to pO2. Using this method over a number of years considerable data was collected regarding pO2 fluctuations in human and animal brains. These studies used frequencies of 0.3–0.7 Hz and were conducted under different physiological situations [11-15]. If +0.3 V voltage is applied to the same platinum electrodes, it is possible to record pH2 as an indicator of local CBF using hydrogen clearance methods.

These recordings of pO2 represent slow fluctuation with related frequencies, and are widely used for the quantitative measurement of local CBF in animal experiments [16-18]. They are also used in clinical observations during neurosurgical procedures [19]. It is very important to note that fluctuations of pO2 and pH2 in animal and human recordings look quite similar.

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Bioimpadence fluctuations

Recording of high frequency electrical impedance (15-100 kHz) between plate electrodes – (1.5–2 cm2 in area), placed on the human head allows the researcher to observe slow fluctuations. These fluctuation measurements are determined by the difference in the electrical conductivities of blood, CSF and brain tissue [20-22]. This method, called Reoencephalography (REG), allows the recording of three kinds of fluctuations – connected with heart activity, respiration and very slow fluctuations. The latter are called waves of the third order. Over two decades considerable data concerning the physiological and medical relationship of fluctuations, primarily related to pulsations from heart activity, has been analyzed. A number of indexes of REG pulse changes have been proposed to calculate the basis of the cerebrovascular parameters [23,24]. However, for a few years, interest in the REG method became non-existent. This was due to the fact that the true meaning of these indices was accurate only for a narrow range of physiological and clinical conditions. Respiratory waves and waves of the third order didn't attract attention from medical scientific investigators due to the problems connected with accurate and timely quantitative analyses.

Over 60 to 70 years other methodologies were developed which could also record slow intracranial fluctuations. These include a group of mini-invasive methods which could be applied to neurosurgical patients – thermography, miniature intracranial pressure transducers, implantation of gold and platinum wire electrodes (100 μ or less) for recording brain oxygen levels and CBF by the hydrogen clearance method [13-15]. All these methods make it possible to record slow fluctuations of intracranial origin.

Background of fluctuations

The methodologies used in different research studies rest conditions and under functional tests in healthy volunteers. In some cases of pathology; as well as in animal experiments, this experimental material provided the basis for certain conclusions about liquid volume fluctuations inside the craniospinal cavity. These studies indicate that intracranial slow fluctuations have multiple sources.

Firstly, there are fluctuations reflecting control processes in the blood circulatory system that are responsible for controlling arterial pressure and activating periodical changes in arterial pressure. These have variations in amplitude of (0.1-1.0 of stroke pressure) and frequencies of 3-15 cycles per minute at rest conditions. There are also slow arterial pressure fluctuations with irregular time durations [25,26]. Volume fluctuations are controlled by periodical pressure changes in the craniosacral systemic hemodynamics. Local fluctuations inside the craniospinal cavity are characterized by a high level of heterogeneity and may be independent even in much localized brain tissue. Some role in the origin of the global volume fluctuations may be due to periodical CSF movements [27]. This mechanism may be responsible for maintaining the metabolic balance in the Central Nervous System for all body organs. CSF's role in organizing the function and structure has only been investigated recently [28].

Secondly, changes in central venous pressure (CVP), controlled by intercostals and diaphragm respiratory muscles movements, and are distinctly different below and above the diaphragm. Cardiac activity also initiates pulsations in the jugular veins; and these controllers interact. Thus the final pattern of fluctuations is a combination of these forces. One more factor, which enters into these fluctuations, is the pattern of pulsations in the cranial and lumbar regions of the spinal cavity itself [29]. Respiratory fluctuations reflected in the central venous pressure have different phases caudally and cranially from the diaphragm movements [30]. Similar to the jelly brain tissue macrostructure this allows the spinal fluid to be freely displaced, [31,32] and may allow CSF replacement in different craniospinal cavity compartments. Total liquid volume fluctuations may be integrated at different cranial and spinal cavity levels with overall liquid volume balance occurring by regional CSF replacement.

Thirdly, there are local periodical slow fluctuations. These have been observed in human studies in patients (with gold wire electrodes 100 μ in diameter and a 1-1.5 mm open insulation tip, and with a distance between them of 3–5 mm, implanted directly into brain tissue). These electrodes allow the recording of slow fluctuations of pO2 and blood volume in small local areas of brain tissue. Slow fluctuations of electrical impedance fluctuations, which correspond to blood volume changes, have also been recorded and displayed. It is important to emphasize that manifestation of these fluctuations may be represented not only by amplitude changes, but also by fluctuations in the amplitude of pulsations which completely disappear for a few seconds. This phenomenon, described some decades ago [22], has been explained about 20-30 years later as the effect of fluctuations of perivascular gaps and changes in volume of extracellular liquid [33-35]. These observations have been confirmed in animal experiments (rat, rabbit, cat), using 30-40 μ electrodes. This research shows the similarity of pO2 and electrical impedance slow fluctuations. The studies also demonstrate the differences between fluctuations recorded with a 0.5-2.0 mm distance between the electrodes [17]. The use of a pH2 clearance method, electrochemical generation of hydrogen in brain tissue of narcotized rats, shows confirms similarity of slow fluctuations received by electrical impedance and pH2 clearance methods Importantly, the frequency ranges of slow fluctuations are in a narrow band for all investigated mammalians – 0.1-0.4 Hz .

Fourthly, the high level of localization of slow volume fluctuations in brain tissue allows us to predict that this type of fluctuation represents processes in brain tissue connected with the brain's functional activity and metabolism. Direct evidence of this is the appearance in some experimental conditions with conscious cats (in a darkened room) of the close correlation between cortex electrical activity (15-24 Hz), electrical impedance and pO2 with frequencies of 10-15 cycles/min in the visual cortex [17]. Human observations demonstrate an increase in the frequency of slow fluctuations as recorded by REG during emotional excitation and evoked stress situations [36]. The fluctuations decreased with decreased brain activity as observed after brain injury [37] and they ceased entirely during narcosis. It was also observed that slow fluctuations did not change during significant vasodilation when the vasodilation was not influenced by sympathetic stimulation or inhibition. Fluctuations decreased during hypercapnia and increased during oxygenation. [14,15,38,39].

Fifthly, some biochemical indices also demonstrate slow fluctuation activity. This was observed in experiments with unanesthetized cat and rabbits. These experiments showed that cortical cytochrome oxidase redox state (CYT) and cortical blood volume (CBV) normally oscillate at 0.4 to 0.5 Hz. These continuous complex oscillations represent fluctuations of the cortical metabolic rate. Their frequencies are varying over time, and indicate interhemispheric synchronicity between distances of 50 mm in 2 cortical regions [40]. This data allows us to conclude that localized slow fluctuations are connected with metabolic changes in brain tissue; and is closely connected with the functional activity of specific local brain regions. It is important to emphasize,

that the resolution between neighboring brain regions may change according to brain functional activity. This conclusion is illustrated by data of cross-correlation analysis of pO2 fluctuations taken from electrodes in associated areas of the brain cortex with distances of 2-3 mm between them, during a 2 minute mental functional test (Raven matrix). Before test the coefficient of cross-correlation between slow pO2 fluctuations is about 0.5– 0.7; during test after 1 min it increases up to 0.7–0.9; after 2 min, at the end of test, is between 0.7–0.8 , and 2 min after finishing of the test this coefficient decreases back to the original test value. Reaction to this test taken from surface (cortex) and deep (white matter) electrodes are different [17].

Categories of fluctuations

Collected data and their analysis shows that slow fluctuations should be divided into three categories from the point of view of initiation:

1) The most global slow wave activity inside the crania – spinal cavity is initiated by changes of indices in the central hemodynamics: systemic arterial and venous pressure, which have periodic components connected with control processes of the blood circulatory system. Some modulations of volume/pressure relations inside crania - spinal cavities may cause periodical global CSF articulatory movements of the skull bones and as a result, changes in the skull dynamics and its internal volume [41].

2) Changes of blood volume internally were evoked by vascular volume changes initiated by periodical processes of the heart and respiratory activities. They basically have regional peculiarities, determined by pressure/flow indices, which are different in magisterial arteries and veins, supplying and removing blood from different regions in cranial and spinal cavities.

3) Vascular and CSF volume changes, which are connecting with the functional activity of the brain and the activity of its different functional structures are based on changes in numerous metabolic processes and, as final result, changes in local brain blood volume and CSF replacements. They represent most perfectly the true conditions of brain function and, therefore, are significant to accurately monitor brain function under different and extreme situations, including measurement of physiological loads during diving and swimming training as well as a number of pathological conditions.

The one and two models have now been investigated many times, as has the analysis of REG pulsations used in practice, but the third model is still unclear. This modeling was of interest to our investigators, mainly due to the absence of knowledge until recent times

Aim of paper

Aim of this paper focuses on the study of slow fluctuations connected brain functioning, which reflects the fluctuations of brain blood volume and CSF and consequently CSF circulation. Recently, REG methodology was applied to healthy persons at rest conditions, during different physiological functional tests and in some cases of pathology. Experiments with awake rabbits have also been conducted. For quantitative analysis of intracranial slow volume fluctuations computerized analysis of spectral analysis of fragments of REG continuous recordings were used to evaluate their spectrum characteristics in ranges of 0 – 0.4 Hz (5–15 cycles per minute), provided under different conditions. For further evaluation of the data received, Transcrsnial Dopplerography (TCD), and respiratory chest movements (Resp.) were also simultaneously recorded.

Methods

There have not been to date direct methods to quantitatively record periodical changes in fluctuations of the liquid media inside closed crania-spinal cavities. In principle, it may be acceptable for these purposes to use MRI methodology. However, the application of MRI is accompanied by some problems (technical analysis subjectivity, absolute subject/patient immobilization, the high price of equipment, difficulties with receiving an accurate data signal for analyze by spectral methods). Therefore for the purpose of this present study it was necessary to select a methodology, which was direct and could provide comparative data dynamically, and also be applied multiple times safely to the same patient. Additionally, it should be relatively easy to use for complex investigations. Rheoencephalography - REG method, in its most modern version was chosen as the most acceptable. REG methodology provides comparatively quantitative spectrum analysis, due to its calibration capabilities, using as a standard unit value of amplitude the pulse spectral line

REG method

The REG method is characterized by a number of important and useful properties. As has been shown by special investigations [14,42], the REG method allows the monitoring of changes in blood and CSF volumes in the brain, where distribution of an electrical field is initiated by electrodes placed on the skin surface of the head.. By varying the electrode placement measurement configuration of the head could be changed. Therefore, REG allows the investigation of different regions of the cranial cavity. REG does not create any biological influence by the electrical current applied and therefore it is possible to provide multiple observations of the same subject.

In this study we have used a new REG modification – Multifrequency (MultiREG), which permits simultaneously recording on three frequencies: 16, 100 and 200 kHz - manufactured by "MISTAR" (Russian Federation). This unit provides information concerning the water content of brain tissue and additional information reflecting intracranial water volume changes. In this study the MultiREG goal was to find the optimal conditions to receive valid spectral diagrams and to establish any factors which could be involved in changing its pattern. All investigations were conducted with fronto-mastoid electrode position for both hemispheres to evaluate spectrum hemispheric asymmetry.

REG and TCD coupling

For purpose of evaluation of CSF mobility, a fragment of recording was selected for spectrum analysis, using the MultiREG, TCD in basement of MCA by "MultiDop" (DWL, Germany) and chest respiratory movements by a specially constructed chest band. By such selection of MultiREG electrodes and TCD probe positions the current distribution inside the cranium includes the entire brain region and separately each hemisphere, which is supplied by blood through the MCA. This allows us to receive comparable data for different subjects and different physiological conditions and to compare the MFREG fluctuations with linear blood velocity in the basement of the MCA.

Animal experiments

Animal experiments were deemed necessary for receiving additional data at two directions to investigate how common results of spectral humans and animals are. Results of such investigations are opening the possibilities to study with animal such particular conditions, which is may be unacceptably in human investigation, for example a few minutes hypoxic condition or to test effect to cerebrovascular control

mechanisms of some drugs. For investigations healthy animal – rabbits have been selected, due to their relative placid nature compared with other laboratory mammalians and to use awake rabbit without any premedication. MFREG electrodes (2 mm x 3 mm) will be placed bilaterally to chemically depilated skin on animal head and softly fixed by rubber bandage. It was possibly to obtain in gently restrained awake rabbits comparatively long (2-5 minutes) a good quality fragments of recordings at rest and under of some functional tests with different respiratory gas mixtures – pure oxygen, hypoxic (7-8% oxygen), carbon dioxide (7-8% CO2 in air). The study was performed in accordance with the Declaration of Helsinki, with institutional ethics committee approval, and all subjects provided written informed consent. All experimental procedures conformed to recommendations of Physiological Section of Russian National Committee on Bioethics and were approved by local Institutional Animal Care Committee.

Spectrum diagrams

Data outputs from all instrumentation were connected with PC "Windows XP" via an analog-digital transformer "PowerLab-5" (AD Instruments, Australia). The PowerLab software provides spectral analysis of all recordings in a wide range of frequency bands. Two spectrum fragments were used: one included frequencies 0.0–2.0 Hz; together with slow fluctuations, pulse and respiratory fluctuations as well as fluctuations which were related to the subject's central arterial pressure. This fragment was used for calculating the value of separate low frequency spectral components by comparing with the REG pulse as a standard signal, and which was designated as 1.0. In rabbit experiments the most stabile and pronounced are respiratory chest movements and their amplitude was designated as 1.0. The second fragment includes frequencies which reflect slow fluctuations of intracranial liquid volume changes. For low frequencies these are limed by fluctuations of the central arterial pressure, which are 0.02–0.15 Hz. For high frequencies these are limited by chest respiratory movements, which are 0.12–0.8. Because frequencies of central arterial pressure and respiratory chest movements limits these fluctuations may vary. In some cases low limits of fluctuations of intracranial origin may be superimposed by slow arterial pressure fluctuations and upper limits may by superimposed by respiratory waves. Therefore, for clarity we compared spectral diagrams fluctuations of intracranial origin with spectrum diagrams of arterial pressure and the respiratory spectrum diagram, calculated at the same frequency ranges 0.1–0.4 Hz (Figure 1) In animal experiments the low frequency component may be superimposed with arterial pressure fluctuation only, because the respiratory rate in rabbits is too high 1.0–1.8 Hz. There are no noninvasive methods to record changes of arterial pressure in rabbits, but indirectly these fluctuations are reflected on ECG recordings (Figure 2), because the basis line and heart rate are connected with changes of arterial pressure. It could give some expression, concerning this kind of slow fluctuations.

From Figure 1 and 2 the quality of spectral diagram may be different and the interval between components depends on frequency of quantification. On Figure 1 it was 1024 points to 1 inch of recording, on Figure 2 – for 1 inch - 124.000 points. Of course there are extreme limits of quantification and for every particular case it is necessary to select two values – length of fragment of recording and rate of quantification depends on the physiologic process under investigation.

Selection of fragment of recordings for analysis.

Significant problem is the selection of fragment of recording for investigations. The fragment should be long enough to reflect accurately the details of the process under investigation. As is have shown on Figure 3 – the length of the fragment for this present study – to investigate peculiarities of slow fluctuation of intracranial origin, and should be about 3 minutes. The fragment should be long enough to reflect accurately the details of the process under investigation. As is have shown on Figure 3 – the length of the fragment for this present study – to investigate peculiarities of slow fluctuation of intracranial origin, and should be about 3 minutes.

Selection of rate of quantization

The role of frequency of quantification depends on quality of the spectral diagram. With the decrease of frequency of quantization the resolution of the analyzed display of the spectral diagram also decreases. With decrease of quantification some details of spectral diagram are missed, but fortunately up to some limits of frequency decrease it is possible to see a general pattern in the spectral diagram. However, with further decrease of frequency it is lost (Figure 4). This shows that the lower limit of frequency of quantification for this particular subject is about 32 k, and when the quality of the spectrum diagram is severely depredated. The data quantification, presented in Figure 3 and 4, show that the results of spectrum analysis and, therefore, its informational meaning, critically depends on the optimal selection of the length of analyzed recording fragment and the frequency of quantification for every particular purpose of investigations. The present study length of the investigated fragment has been selected at 160-180 seconds and quantification 124 k. This limits the length of the functional tests used in our investigations. Their duration should definitely be longer than 3 minutes and during all investigations the subject under investigations should be at rest and passive. In some cases, depending on the particular purpose of the investigation and its particular condition, it is possible to

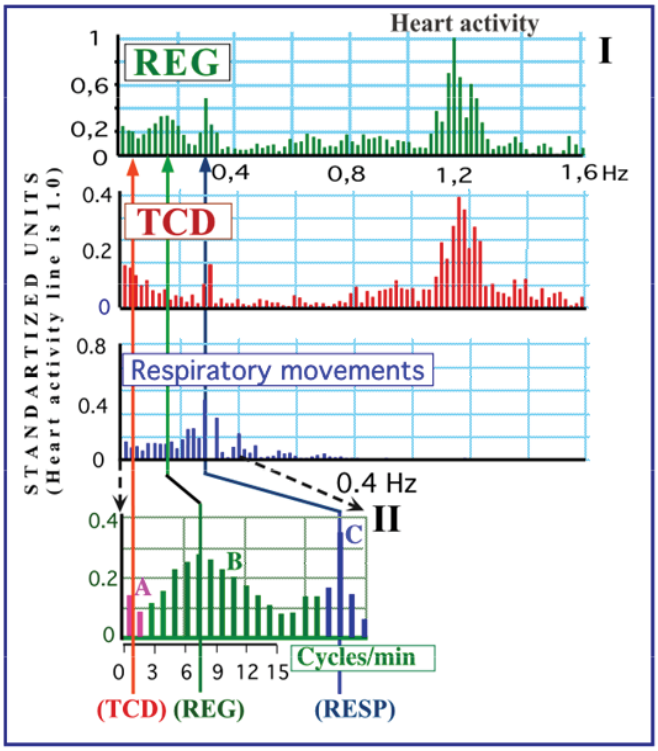

Figure 1 Spectrum of REG in ranges 0–1.6 hz, (i) which includes all types of slow fluctuations (ii) demonstrates fragment of spectrum, which belongs to slow waves of intracranial origin(a) surrounding spectral lines of arterial pressure(a) and chest respiratory movements(c).

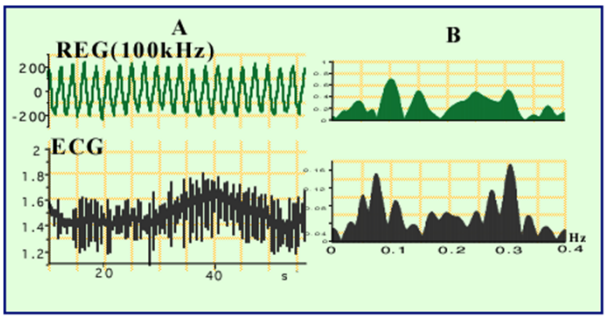

Figure 2 Original recordings of REG from electrodes, placed bi-temporally on a rabbit head with chemically removed fur and ECG, taken at the 2nd standard position. On the upper curve the large amplitude fluctuations are respiration and the small, modulated are heart pulsations (a). ECG recording reflects besides "r" peaks of ECG also deviation of the base line, which reflect indirectly the fluctuations of arterial pressure, depending on heart rate.

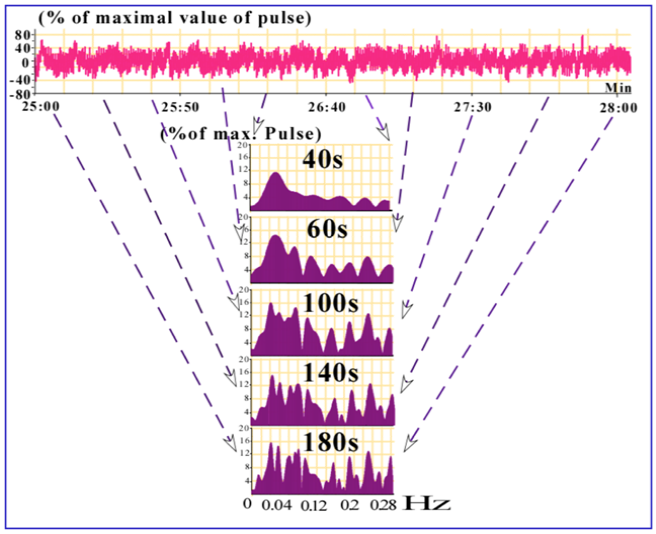

Figure 3 Comparative results of spectral analysis of different fragments of recordings from 180s to 40s.

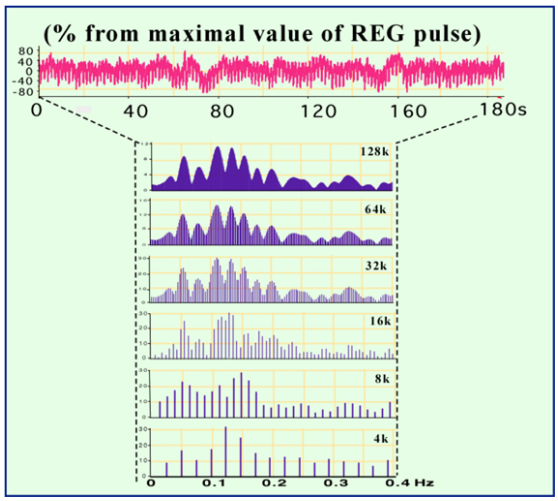

Figure 4 Effect of decrease of frequency of quantification in the pattern of the spectrum diagram.

use a shorter fragment and less quantification, but the possibility of loss of information should be taken into consideration.

Materials and procedure of investigations

Investigations were conducted on 27 healthy volunteers of both sexes with age ranges of 17-25 years. The data were collected at rest, during short time tests (apnea, hyperventilation, Stookey test) and long period test - tilting head down 17o up to 30 min., as well as in experiments on 12 rabbits. Investigations were conducted with 27 healthy volunteers of both sexes with age ranges of 17-25 years. Data was collected at rest and for long periods (tilting head down at 17o) for up to 30 minutes. 12 rabbits were used for the animal experiments. Specific functional tests were selected for the best quality spectrum analyses, as it requires fragments of recordings at steady state conditions. The subject's system under investigation normally adapts to changed physiological conditions in about 3 minutes. Observations how, that spectral diagrams are very changeable and it is no reasons to apply statistical methods for their analysis. Indeed, spectral diagram in frequency interval 0-0.3 Hz include number of peaks of spectral lines but their position is vary. If they will be averages, individuality of peaks will disappear. Investigation with 27 persons show, that for all of them spectral diagram is similar, but positions of particular peaks may vary. This permits to conclude that low frequency spectral diagram is similar for healthy persons, but not the same. It is necessary in future to develop quantitative methods for evaluation of informational meaning of low spectrum peaks. It is possibly to provide by two ways – to look for correlation between particular the special lines and other physiological indices and to look for correlations of particular spectral peaks on recordings, provided on different frequencies of REG recording under different conditions, for example, functional tests. However, investigations with functional tests, used at this study takes to provide about 3 min recordings just before test – for evaluation of spectrum diagram at rest conditions, then recordings during the functional test, also about 3 min and the same fragment of recording for post test period. The recordings are necessary to evaluate influence of functional tests.

Results

General suggestions

Based on our results from the present investigations and previous research investigations of intracranial liquid volume changes, fluctuations are best studied and measured by high frequency (15-200 kHz) impedance methodology. This method more accurately measures the changes in the liquid media in the cranial cavity when electrical current is passed between electrodes placed on the human head. By varying the electrode position it is possible to change the current distribution inside the cranium and therefore the cranial region to be investigated. Taking this into account in all human impedance recordings, the bi–fronto-mastoid MFREG electrode positions were selected. We reviewed the results of spectral analysis in the frequency ranges of 0–0.3 Hz, with 180 second fragments of MFREG recordings. This frequency band included slow fluctuations in both human and animal subjects [43]. Recordings fragments should be selected without artifacts and recorded on a 16, 100 and 200 kHz current frequency. The middle frequency – 100 kHz is commonly used in impedance investigations and comparing it with data received at 16 and 200 kHz it is possible to investigate current distribution between intracellular and extracellular spaces. The analyzed displays are very close. Heart pulsation and chest respiratory movements are represented in both spectral diagrams, but due to different heart and respiratory rates they display differently. However, the narrow spectrum components

of humans and animals look very similar. This gives support to suggestions that spectrum components of humans and animals, which are not connected with heart activity and respiration, have a similar physiological background. The last observation is important not only from a physiology point of view, which indicates the similarity of metabolic processes in the human and animal brain, but that these waves represent the liquid volume slow fluctuations of intracranial origin. They also offer the opportunity to study these fluctuations using data collected non-invasively from human and animal investigations. This significantly expands the possibilities for studying this physiological phenomenon, because it provides data from either human or animal observations. This type of study can use different physiological conditions and record determined physiological indices, some of which it is possible to observe with humans, but others only in animal experiments.

The study of the structure of the spectral diagrams at rest and under different physiological conditions indicates that we deal with two types of investigations. One is based on the comparison of spectrum components of MFREG with the spectrum components of other physiological processes of similar frequency also at rest conditions. The second is based on comparing spectral diagrams of recordings, taken under changed of physiological conditions, which could be help in understanding the background slow volume fluctuations inside the cranium. It is more convenient to collect data from human observations in one case, in the other animal experiments, based on the above mentioned fact, concerning the similarity of low frequency spectrums in human and animals (rabbits), which allow us to predict that these slow fluctuations have a common origin in mammalians with a brain metabolism close to that of humans.

Current results

The comparing of spectrum diagrams of slow fluctuations with indices in human persons at rest for about 180 seconds fragments of simultaneous recordings of MFREG, (recorded on three frequencies – 16, 100 and 200kHz), together with TCD and respiratory fluctuations are presented as spectrum diagrams in Figure 5: Firstly, it is necessary to mention that the MFREG spectrum, taken from the left and right hemispheres, generally look different and some lateral peculiarities are clear. For example, the general pattern of the spectral diagram of the left and right hemispheres looks dissimilar. Some details are of the spectrums and their relations to the spectrum diagrams of TCDG and respiration, obtained on different REG frequencies (this is have shown by arrows at Figure 5). Some additional groups of spectral lines appear to come from only one hemisphere. There are also other differences in the displayed analysis, which belong to only one hemisphere. Comparing the low frequency spectrum of the MFREG recordings at different frequencies sees this.

Hemispheric asymmetry

The current distribution between the electrodes, fixed on the human head is different due to the frequency and electrical conductivity of the liquids and tissue contained in the cranium. It is possible to see this in Figure 5, by comparing the MFREG spectrums recorded on different frequencies. This shows that there is left/right hemispheric asymmetry of slow fluctuations, as was observed earlier with hemispheric asymmetry of pulse CSF mobility and Cranial Compliance [44]. The similarities and differences of the peaks of dynamics in physiological processes depend on a number of factors. It also indicates once more that the crania-spinal space, filled with liquids, has some structural peculiarities responsible for the difference of hemispheric CSF mobility, hemispheric cranial compliance and as a consequence of all of this – the hemispheric difference in the spectral diagram of liquid volume fluctuations.

For groups of peaks - red arrows - the most pronounced low frequency peak belongs to TCD, which accompanies the MFREG wave. The second peaks of the MFREG are in most cases larger than the TCD peak and look different at both different frequencies in each hemisphere. This indicates that the value of the second peak of MFREG depends on some other physiologic process.

Peaks of spectrum belong basically to MFREG and may vary, but not significantly. The limits of variations of MFREG peaks at rest conditions are 0.3–0.7, which were compared to the value of heart pulse of MFREG, measured on a corresponding frequency and taken as 1.0. Comparison of Spectral components of MFREG, recorded using different frequencies, show that they have some differences, most pronounced if compared to MFREG spectrums taken on 16 and 200 kHz and MFREG on 100 kHz. Where hydration of the brain may take place, both 16 and 200 kHz REG spectrums will be demonstrated, but only one REG spectrum on the 100 kHz frequency is shown in our analysis/display.

Variation of spectral diagram

Respiratory changes are dominant on the spectral diagram and MFREG spectrum diagram at higher than 0.3 Hz and usually correspond to the spectrum diagram of respiration (see green arrows on Figure 5). Therefore, it is possible to say that below 0.1 Hz and higher than 0.3 Hz REG spectrum diagram generally depend on extra cranial factors. Between these limits physiologic processes of intracranial origin, as reflected by MFREG spectrum analysis, determine liquid volume fluctuations. Significant information could be received from analysis of changes in Spectral MFREG, recorded on three frequencies. TCD and respiration component changes during functional tests, which are directed to the cerebrovascular and CSF systems, also provide valuable information. However, take into account that to achieve a good quality low frequency spectrum diagram, as in Figure 3 and 4, it is necessary to have for analysis about 180 seconds of continuous recording, practically without interference. Significant changes in spectrum diagrams can be observed by comparing data, received with aging subject (Figure 6) there are some common spectral peaks for all investigated subjects, but the maximal value of the spectrum diagram is different. With age the general value of peaks on the spectral analysis diagram decrease. This may be connected with a decrease in activity of some brain metabolic control mechanisms.

Functional tests

The most significant for the study of low frequency slow fluctuations is to evaluate their changes under different physiologic conditions. For this purpose functional physiological tests are used. However, in brain circulatory physiology a short period of time - about 30 seconds in duration of a functional test - We used Stookey and Valsalva tests, apnea and hyperventilation. However, from the point of view of methodology for low frequency spectrum analysis it is necessary to take for analysis fragments of recordings of about 3 minutes to evaluate the majority of peculiarities of the spectrum diagram in frequency ranges of – 0.3 Hz. Most of aforementioned functional tests are not valid for long term use, and the orthostatic test with head down tilt could only be applied for a period of number of minutes (results are shown on Figure 7.) It is possible to see on Figure 7, which after 7 min. from the beginning of tilting the amplitude of spectral lines increases at 16 kHz, but decreases

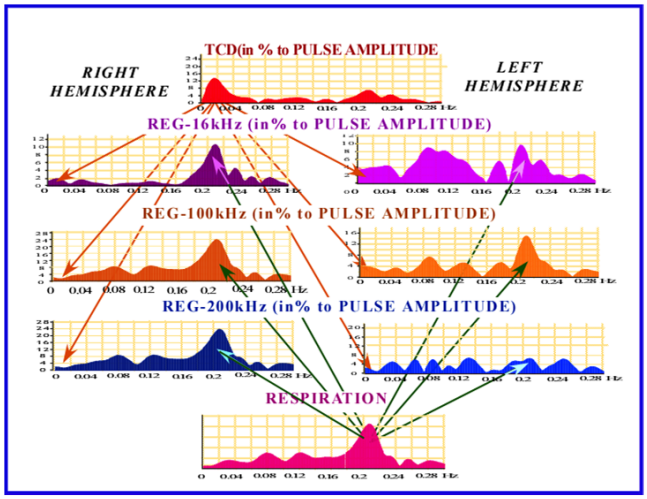

Figure 5 spectral diagrams taken simultaneously with TCD (basement of mca), REG on 16, 100 and 200 khz (fronto-mastoid electrode position and respiratory chest movements at rest conditions of healthy 27 year old person. Quantification 128 k.

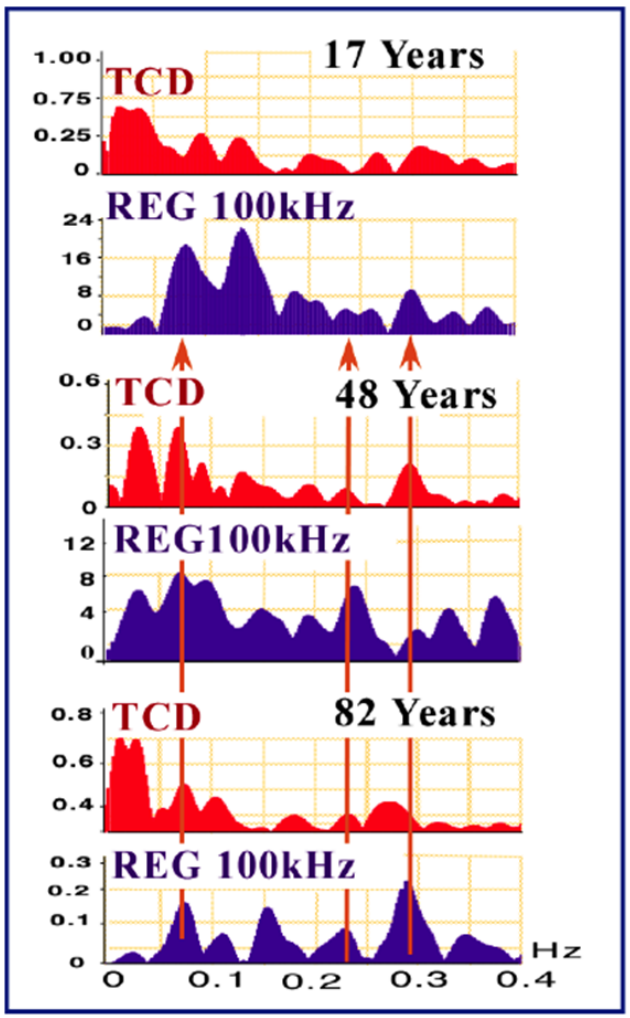

Figure 6 Groups of TCD and REG (100khz) recordings, which were taken with investigations of healthy persons of different age in a comfortable resting horizontal body position.

at 100 and 200 kHz. This indicates that some redistribution between blood flow control inside and outside of skull has taken place.

Comparative changes of MFREG and TCD

The changes of TCD spectral diagrams on Figure 7 also change but not corresponding to the MFREG changes. This data shows that the increase of blood pressure in the upper Cava vein evokes changes in the spectrum diagram, reflecting liquid volume fluctuations inside the cranium. It requires the specific study of identifying features to determine the physiological meaning of each spectral peak on the REG analysis diagram, though we are now realizing the importance of this information.

The possibilities for long-term functional tests in experiments with animals are much more feasible. It is possible to apply hypoxic (about 5-7 % of oxygen in area), hypercapnic (7-10 % CO2 in area) and hyperoxic (about 100% O2) functional tests. These tests have been used for evaluating changes at low frequencies evoked by functional tests to provide similar measurements in healthy adult rabbits (2.5–3.0 kg) under non-invasive conditions. For MFREG recordings two plate electrodes (2 x 4 mm) were fixed bi-occipital on the skin of the animal's head (Fur had been chemically removed) Together with two frequencies of MFREG, the rate of respiration and ECG was measured. The overall condition of the animal under these conditions showed that the low frequency spectrum of MFREG, demonstrated above, is similar to that of a human. However, the differences between data obtained at different REG frequencies is not so pronounced in rabbits as in humans, perhaps due to the comparatively thin skull bones of the investigated animals. Therefore, experimental measurements were limited only to data recorded using 16 and 100 kHz frequencies.

The data presented in Figure 8 shows significant changes in response of spectrum diagrams to changes of the respiratory gas mixture, This is due to ability to measure long-term changes especially comparatively long were hypoxic and hypercapnic tests. It was shown that after a period of adaptation to the applied functional tests (6-7 min after their start), some definite changes in the spectrum diagrams of 180 second duration REG recording could be observed. After pure oxygen inhalation the line values, as represented in the spectral diagram, basically decreased between 20-50 %, particularly at frequencies of 0.15–0.25 Hz. Inhalation of hypoxic gas mixture is accompanied by some increase of line values (30-35%), displayed in the spectral diagram by a decrease in the number of peaks in the analyzed spectrum interval. During inhalation of 7.5 % of CO2 the area value of spectral lines increased from 20-45 %, especially in frequencies of 0–0.2 Hz. The data showed that inhalation of the above described gas mixtures evoked definite changes in the low frequency spectrum, different for each of the tested gas mixtures. The reason for these changes in the low frequency spectrum diagram may be based on the influences of respiratory gases on brain tissue metabolism and also direct influences on the control mechanism of the brain circulatory supply. Physiological data support both of these.

Discussion and Conclusion.

All the above data and diagrams definitely show that the use of spectral analysis of slow liquid volume fluctuations is a powerful method for the quantitative measurement of fluctuating physiologic processes with changes in ranges of wave amplitude and frequency. Fluctuations at different frequencies, mainly of low frequencies below that of the heart activity and respiratory chest movements, and not of an electrical origin, accompany numerous physiological processes; particularly in

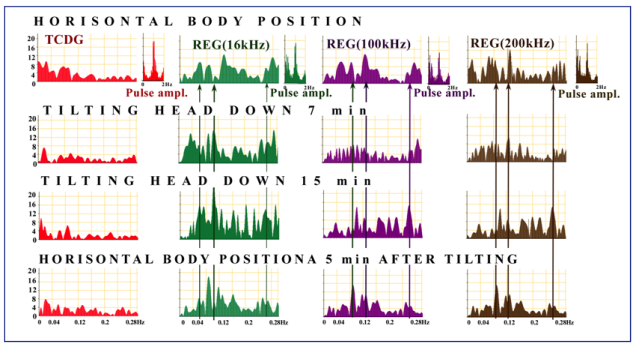

Figure 7 Changes of low frequency spectrum in a healthy subject 25 years old before, during tilting head down (17o) and 5 min after returning to a horizontal position. Arrows show expressions of the same frequency peak in the spectrum diagram during the development of an adaptive reaction to tinting and recovery afterwards. Small size spectral diagrams in ranges 0-2 hz upper-left indicate the value of pulsation for calibration and low frequency spectrums. Quantitation - 128 k.

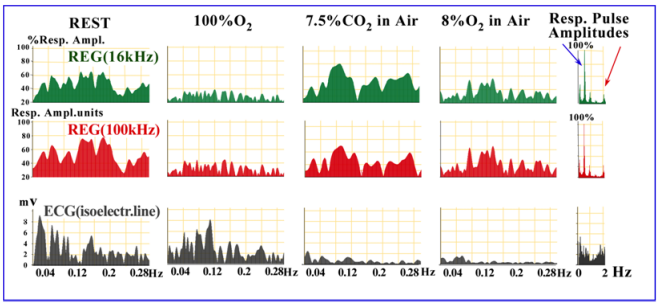

Figure 8 8 Changes of low frequency spectrums of REG (16 and 100 khz) and isoelectric line of ECG at rest and during long term different gas inhalation (100% O2, 7,5%CO2, 8% O2). For analysis fragments of recordings of 180 s to nearly10 min duration were taken.

the functional circulatory systems and in the relationships between them. The phenomenon of slow fluctuations has been known for more than a century. Until the present time, studies of slow fluctuations were mainly conducted occasionally, and didn't form an informational index in physiology or medicine. At that time only one branch of medicine – Osteopathic Medicine - was interested in slow fluctuations of an intracranial origin. However, the absence of instrumentation for the quantitative evaluation of slow fluctuations only allowed evaluation by osteopathic manual methods. A mathematical method for this purpose, the Furrier analysis, was then developed.

In the 21st century, fast and simple computer methods for the calculation of the spectral diagram have been developed. The data presented above is one of the first investigations of intracranial liquid volume fluctuations. Of course, it was impossible to give a detailed explanation of all the analyzed features in the low frequency spectrum. However, it is possible to conclude that the frequency ranges for slow fluctuations of intracranial origin are limited generally to 0–0.3 Hz. It is important, for representatives of mammalian species – rabbits – that frequency limits for slow fluctuations of an intracranial origin are nearly the same as for humans. It is now possible to study slow fluctuations, using experiments with mammalian animals.

It is also now possible to determine a number of factors, which could be responsible for the intracranial liquid slow fluctuations. It is necessary first of all to remember that intracranial liquids are a passive media with no internal forces for motion. The liquids could however move when external forces are applied. From this viewpoint, for intracranial liquid volume slow fluctuations a major source of force is the cerebrovascular system; particularly different local levels of changes to vascular tone due to changes in the functional activity of brain tissue, change in brain metabolic processes, and changes in the general circulatory and respiratory systems. All these factors are reflected in the spectral diagram of intracranial liquid volume slow fluctuations. The most interesting aspect of these studies was to establish correlations between the amplitude of particular spectral lines and some indices of brain metabolism. Our recorded data indicates that intracranial low frequency volume fluctuates in ranges of approximately 0–0.3 Hz and reflects general brain metabolic processes with some of their peculiarities. Spectral indices of liquid volume fluctuations and their spectral diagrams may also reflect their quantitative features, which may be significant for neurophysiology and clinical applications.

This research data indicates that it is possible to observe a correlation between brain volume slow fluctuations and its cognitive function. Support for this statement could be investigations of brain blood volume fluctuations of astronauts during acceleration testing [36], which demonstrated an increase of amplitude in slow fluctuations related to emotional load. Comparison of spectral diagrams for persons with age dependent decreases in cognitive function in middle age (45-55 years) and elderly (after 75 years with symptoms of dementia) with brain circulatory insufficiently [45], show that cognitive dysfunction of some of these subjects compared with healthy people as determined by psychophysiological "Prognosis" method [46] is a general decrease in amplitude and position of some groups of spectral lines. This data demonstrates that slow brain volume fluctuations closely connected with a change of brain function is both a metabolic activity and a cognitive function.

This current research shows that intracranial slow volume fluctuations in frequency ranges from 0.0 to 0.3 Hz reflect complicated physiological processes in brain tissue and changes in the brain circulatory system. The spectrum diagrams display a quantitative measurement of these processes. It is important to take into account that the quantitative representation of slow periodic fluctuations of an intracranial origin is a prospective approach for the study of the control processes responsible for the circulatory-metabolic support of healthy brain function. The complex character of the low frequency spectrum, reflect intracranial processes that include a number of single control links and structural components and a comparison of low frequency spectrums under different experimental conditions and models of pathology. This methodology has two important advantages. The first is the possibility to compare human and animal physiological models. This is significant, because some issues are only possible to study in animal experiments, while others – require human observation and common aspects for comparing results. This is now possible with low frequency spectral analysis. The second possibility is to study simultaneously the physiological control mechanisms characterized by particular slow fluctuations and at the same time associated biochemical processes fluctuations. It is necessary to provide the specialized computer analyses for identification of spectral lines, which is different for different physiologic processes, but these results look very encouraging. One possible way may be to base this on a comparison of low frequency spectrums of intracranial origin at rest, with the same subject, with a spectrum of other fluctuating processes taken in different experimental conditions.

Thus, spectrum analysis opens the way for a new noninvasive methodology for investigating the complicated physiological processes; responsible for the brain functioning and the mechanisms that control brain metabolic supply. This could clarify treatments aimed towards healing brain dysfunction with the possibility that the spectrum approach used in this study of low frequency intracranial fluctuations. It looks real, that these changes may provide definitive results, indicate initial functional changes of serious pathology. Described above spectrums of slow fluctuations are similar to that of the applications of light spectroscopy to chemistry and physics. So, that is one of directions of wide spectral methodology, already used at the some branches of natural sciences. This technology may also demonstrate new perspectives for application to modern biophysical questions, based on the discovery of presently unknown control systems not only inside cranium, but in other areas of the body.

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