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Quantitative Analysis of Intracranial Volume Slow-Wave Fluctuations

Yu. E. Moskalenko* Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russian Federation ; Russian scientific Research Center of Fundamental Osteopathy, St. Petersburg, Russian Federation

T. Vardy Queensland University of Technology, Brisbane, Australia

M. A. Sabirov Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russian Federation

T. I. Kravchenko Russian scientific Research Center of Fundamental Osteopathy, St. Petersburg, Russian Federation

N. A. Riabchikova Lomonosov Moscow State University, Russian Federation

N. N. Uglova Russian scientific Research Center of Fundamental Osteopathy, St. Petersburg, Russian Federation

Abstract: This paper describes the results from the application of spectral analysis to the interpretation of slow volume fluctuations inside the intracrania-spinal cavities, recorded during 3 minute data collection periods by a rheoencephalography (REG) method, using a bio-impedance current frequency of 100 kHz and a medial fronto-mastoidal electrode sending position. The collected data was processed with Chart 5 software to obtain the spectra for each recording. Participants were from three age groups 20-30, 30-45 and older than 45 years. 20-30 subjects from each group were investigated, as well as a number of subject patients with neurological pathology, treated with an osteopathic manual technique. 5-7 spectral peaks were revealed in the 0 – 0.3 Hz frequency range. Hemispheric asymmetry was observed in every age group after mean averaging analysis. In the cases of neural pathology the osteopathic treatment intervention changed the amplitude and frequency of the spectral peaks. This study's results suggest that the application of spectral analysis to slow volume fluctuations may provide information in evaluating the integrative relationships of the cerebrovascular system, cerebrospinal fluid (CSF) mobility and cranial pulse compliance. This has implications for non-invasive measurement of the rate of brain function in neurologically normal and non-normal neurological states in humans.

Keywords: Intracranial slow volume fluctuations; Rheoencephalography; Spectral analysis; Hemispheric asymmetry; Osteopathic manual technique.

1. Introduction

Slow-wave volume fluctuations (SVFs) inside the cranial cavity have attracted the attention of scientists for more than 100 years. However, up to the present time data about the nature and characteristics of the SVFs has been controversial, lacking a basis for quantitative evaluation. The initial stage of investigation of SVFs was based on observations of the open skull. A section of skull bone was replaced hermetically by fine glass. Injection of a quantity of contrast liquid under this glass demonstrated the presence of periodical slow irregular fluctuations of cerebrospinal fluid (CSF). These fluctuations were characterized by changes in amplitude and frequency, related to the diastolic CSF fluctuations. A phenomenon of cranial bone motion was observed by manual palpation with a frequency from 5 to 15 cycles per minute. Certain neurological disorders were identified during an initial period of the study of SVFs by Sutherland [1]. Sutherland's palpatory method provided a manual diagnosis of a number of neurological conditions. Osteopathic medical practitioners have utilized Sutherland's treatment techniques from the early 20th century up to the present time.

The next evolution in the study of SVFs was based on electrical recordings of changes in volume and pressure of CSF by insonating special sensors within the cranium. Applying various instrumentation to study SVFs inside the cranium has been documented in previous experiments using implanted microelectrodes and pressure sensors in different regional structures of the brain with animals [2] and in the human brain [3]. These investigations showed that SVFs are associated with a number of brain processes of a non-electrical nature. Cerebral blood flow and cerebral blood oxygen concentration in the brain tissue, as with CSF, vary in different regions of the brain. It was

*Corresponding Author

established that in a minimal region of brain tissue (less 1-3 mm^3) there was a difference between SVFs and that this was localized. These experimental outcomes were established by implanting microelectrodes into the whisker cortex region in a rat brain [4]. It was also demonstrated that these slow wave fluctuations could also completely traverse the brain tissue within a human brain hemisphere [5]. The frequency of fluctuations in both animals and humans varied mainly between 5 to 15 Hz and was sensitive to different physiological perturbations. These studies indicated an associated physiological significance of the SVFs in the cranial cavity. In principle this could be used for diagnostic and for treatment purposes. Thus at the end of 20^{th} Century it had been demonstrated that SVFs are directly connected with the physiological functioning of brain structures. However, while providing abundant data, the analysis of this information was difficult and time consuming. Ungald [6] in his dissertation at Scharite Hospital in Berlin, Germany, concluded that intracranial slow fluctuations have no theoretical or practical significance due to the inadequacy of the available analyses at that time.

The possibility of quantitative analysis of SVFs commenced at the end 20^{th} century due to the development of microelectronics and enhanced computer software programs. This paved the way for the use of spectrum analysis for slow periodic processes such as intracranial SVFs with frequency ranges of 0.01 to 0.3 Hz. The aim of the present study was to adapt the spectral analysis for quantitative evaluation of these slow periodic fluctuations.

2. Methodology

SVFs within the skull are important for a number of physiological processes; intracranial circulation of the CSF and between the cranium and the spinal cavity, intracranial and extracranial forces which create pressure gradients (which changes continuously in different regions of the craniospinal cavity), the motility of the skull bones, and fluctuations in cerebral blood and CSF volumes. The amplitude of the SVFs is often small (about 10% of the baseline values of the studied cerebral factor, but in some cases (disease) they may be significantly higher. An example is the phenomenon of Quincke where arterial pressure (AP) oscillations and intracranial pressure (ICP) fluctuations become very large. Particularly under normal and abnormal physiological cranial conditions, SVFs can provide significant information. Technically however, it is difficult to amplify such small and slow wave signals. Therefore, we considered another way to check slow wave processes within the cranium. This method used the principle that the modulation of the studied oscillatory processes are associated with another process that is also oscillatory, but differs by several orders of magnitude in frequency. Saturation of blood oxygen can be measured by modulating the light signal used in oxygenometry. Periodic oscillations of blood flow velocity can be modulated by an ultrasonic beam, thereby isolating the slow wave changes in cerebral blood flow. In the present study we used the results obtained by modulation of a high frequency current passing through the cranium, and slow wave changes in intracranial conductivity due to the change in volume ratios of blood and CSF. Blood and CSF have different conductivity values. So the SVFs were modulated by the selected electrical current with a frequency of 100 kHz. The measurable difference between the frequency of the modulating current and the carrier signal allow measurements to be made accurately and simultaneously at different frequencies. Measurements can then be achieved for the difference in the spatial distribution of the fluids within the cranial cavity i.e. blood, CSF and water.

We used the 100 kHz frequency because this frequency of rheoencephalography (REG) or bio-impedance most accurately measures the change of volumes of blood and CSF within the cranial cavity i.e. skull. However, REG cannot measure cerebral circulation, because there is no direct correlation with changes in electrical resistance for cerebral circulation as a stand-alone measurement [7, 8]. REG has been used previously for measurements of cerebrovascular reactivity [9]. The use of REG for quantitative evaluation of SVFs may potentially provide a new practical application for bio-impedance. If the electrical gain and SVFs occur simultaneously at high frequencies and with sufficient duration (>3 minutes), interference and artifacts in the spectral analysis of the REG recordings are significantly reduced. Also, changing the base frequency of the probing current changes the penetration depth of the probing bio-impedance signal in the cranial cavity which enters the cranium and flows through all the cranial fluid contents including the brain tissue. REG can be combined with other measurements such as transcranial Doppler (TCD), which records fluctuations in the velocity or flow of blood into the cranium by insonating the middle cerebral artery (MCA) supplying each brain hemisphere. Other cardiovascular measurement systems used in parallel were electrocardiogram (ECG) for heart rate and a chest respiratory band for the subject's respiratory rate. As the volume of brain tissue through which the SVFs flows varies widely, for our study we chose to take a rather extensive area of the brain e.g. a significant portion of the hemisphere supplied with blood by the middle cerebral artery (MCA), by focusing the TCD sensor beam on the anterior parietal portion of the MCA.

3. The Method of Obtaining Data

This was a combination of the REG and TCD technologies, as based on previous studies [10], this has been shown to be the most effective measurement combination. This is essentially because REG correlates with blood and CSF volume fluctuations inside skull, and TCD with the intracranial pressure (ICP) changes by measuring the pulsatile index (Pi). Cerebral artery pressure is related to the volume of blood in the large arteries at the base of the brain. A change in CSF volume, surrounding these arteries, changes the ICP. REG and TCD recordings were carried out using a multi frequency REG (Mf-REG "Mitsar", Russia), and dopplerography recordings were obtained with the 'MultiDop-T' (DWL, Germany). The signal from the sensors was digitized using ADC "PowerLab-8/30" (AD Instruments, Australia), with a recording sampling rate F_d equal to 100 Hz. While data was being recorded the

subject was at rest in a supine position with eyes closed. For analyses we sampled 3-4 minute fragments of wave track recordings. Six (6) REG channels (waves) corresponding to 16, 100 and 200 kHz can be used with this equipment array with both human brain hemispheres, but in this study only the 100 kHz frequency was used together with a single probe channel (wave) from the TCD device for each hemisphere. The digitized data of individual REG registrations was normalized by the maximal values of systolic pulse for each subject. Digital recordings of the wave oscillations were used to build spectrograms of selected fragments in the program Chart 5 (Windows 7). The analysis of spectra of Mf-REG was conducted in the area of greatest SVF value, at a current frequency of 0.01-0.3 Hz, in accordance with previous research in this area [11].

The spectrograms of the SVFs were used to search for and analyze peaks with matching frequency in the different study subjects, to determine if there were characteristic features for different age groups, and to assess possible hemispheric asymmetry in the oscillations. 114 subjects were investigated to provide age-related comparisons. The subjects were divided into three groups: younger (10-30 years, 35 male and female), middle-aged (30-45 years, 45 male and female) and older ageing subjects (>45 years, 34 male and female). For the evaluation of SVFs when providing osteopathic manual treatment techniques with subjects displaying cerebrovascular measurements outside an expected normal range, measurements of individual subject SVFs before and after osteopathic treatment were carried out.

4. Optimal Settings for Spectral Analysis

In many programs using spectral analysis one can identify several key settings which will significantly affect the results. Among the main parameters that may influence a spectral analysis there are four (4) most important: (1) the number of data points in the signal sample (the sample size) (2) the FFT (fast Fourier transformation) value (3) the "window functions" presets and (4) the extent of overlapping of FFT blocks in the process of applying the FFT algorithm.

A detailed and rigorous mathematical presentation of the FFT algorithm can be found in the work of Nussbaumer [12]. In our study, it is sufficient to note that the FFT allows you to convert the signal presented in co-ordinates "time-amplitude" to co-ordinates "frequency-amplitude" to obtain the spectrum of the signal. The higher the value of the sample FFT in the program settings, the higher the resolution of the spectrum frequency. This also depends on the bandwidth used.

$W = F_d / (N_{FFT} / 2)$ where:
W – width of the frequency band in the spectrum;
F_d –sample rate of the recorded, Hz
N_{FFT} – FFT size value

For example, when choosing the number of FFT = 8K, the algorithm will split the array source signal into 4096 frequency bands (1K = 1024). Thus, the step of frequency bands at F_d = 100 Hz will be approximately 100 / 4096 = 0.024 Hz. We did not analyze the whole range of 0-50 Hz, but only a small part = 0.01 – 0.3 Hz. Frequency bands with FFT = 8K are too large (about 8% of the width of the range of interest). To reduce the band-width we had to increase the number of bands by increasing the FFT size value. Thus, when FFT = 32K the bandwidth is 100 / 16384 = 0.006 Hz (or about 2% of the width of the range of interest). This was sufficient for the goals of this study. At a constant sampling rate, increasing this parameter provides a suitable corresponding increase in the number of frequency bands and a proportional decrease of the bandwidth (table 1). This parameter increase also increases the resolution of the spectrum in frequency, but reduces the accuracy of the amplitudes of the peaks. The latter is due to the fact that the FFT algorithm involves averaging the data within a single frequency band, and thus the more frequency bands the lower bandwidth, and therefore the sample points within the band available for averaging.

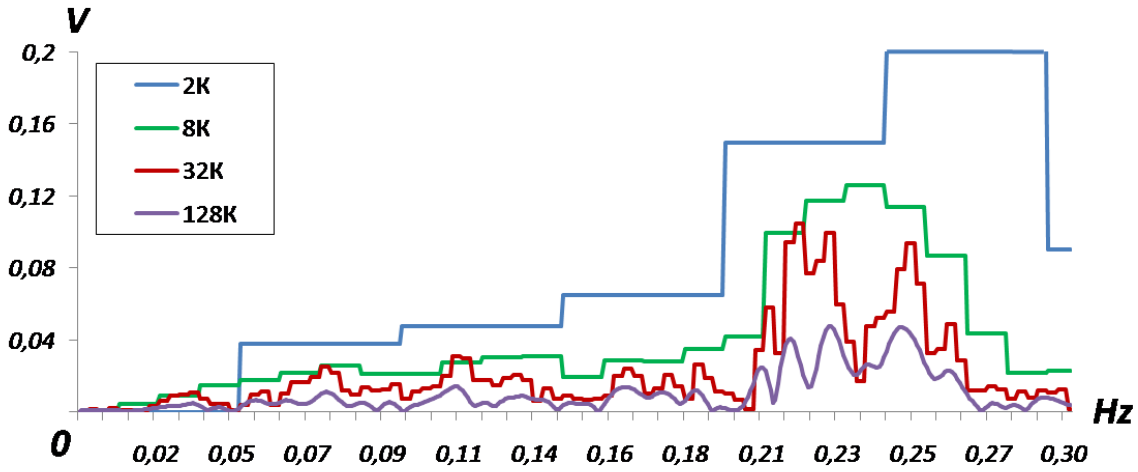
Table-1. Values number of frequency bands and bandwidth for some of the configuration options from the FFT. The Bold settings' values are suitable for the frequency range of 0.01 – 0.3 Hz and at these resolutions provide an acceptable accuracy.

FFT standard setting	**Exact FFT value**	**Number of frequency bands**	**bandwidth**	
			In range 0 - 50 Hz	**%from range 0.01 – 0.3 Hz**
8K	8192	4096	0.012	4.07
16K	16384	8192	0.006	2.03
32K	32768	16384	0.003	1.02
64K	65536	32768	0.002	0.51
128K	131072	65536	0.001	0.25

The decrease in bandwidth by increasing the number of frequency bands leads to an important practical outcome when working with spectral data i.e. the more accurate the frequencies are displayed (a larger number of frequency bands) the smaller the number of signal data points that are necessary per band of the resulting spectrogram. Thus the data representation in the form of the spectra is a compromise in accuracy. The higher frequency resolution (FFT size value) results in a possible error in the view of the signal amplitude (height of the wave). The task of any

researcher is to find the optimal value of the FFT in accordance with the objectives of the specific study. Fig. 1 shows the resulting spectrogram with four FFT size settings (2, 8, 32 and 128K) for the frequency range of 0.01 – 0.3 Hz and the 4-minute fragment of the original signal. With the increase in the FFT size value the amplitude of the peaks decreases while the frequency resolution increases.

Fig-1. Comparison of spectrograms for the frequency range of 0.01 – 0.3 Hz for different FFT size values. In all cases, the duration of the analyzed recording fragment was 4 min.



It is desirable that the FFT is a multiple of the number of pixels (data points) of the original digital signal. Many software programs implement algorithms for spectral analysis and add an extra series of zeros to the end of the digital signal array. It is necessary to equalize the array of initial signals to a multiple of the integer number of a selected FFT size value because the FFT algorithm is usually designed to work with arrays of the size of 2^n. In our case a 4 minute segment of a digital signal with 100 Hz sampling rate contained 4*60*100 = 24000 points (sequential amplitude values in time). The nearest value of the standard FFT size value (table 1) exceeding this amount of pixel points is 32K. When FFT = 32K was selected the source signal added 8767 points for a value of "0". Then the total number of points becomes 32767. If you select FFT = 32K the array will be divided into 16384 frequency bands that displays and analyzes 2 data points of signal per band. Given the above described compromise on accuracy between frequency and amplitude resolution, it is not always reasonable to strive for the highest possible values of FFT size. This can be justified by the important role the maximum possible frequency resolution plays, and in relation to the recordings, obtained with a high F_d.

Two other parameters have a significant influence on the results of spectral analysis. The "window function" and the degree of overlap of the FFT blocks. The first is used for smoothing the initial and final parts of the original recording signal. In the absence of such smoothing, artifacts in the form of peaks at high frequencies may appear in the spectrogram. This can occur because the analyzed fragment is finite, whereas one of the theoretical assumptions of the FFT is the infinite duration of the analyzed signal. In practice, no signals are infinite, and their endpoints have a gap. The essence of smoothing a multiplication of the analyzed fragment is to reduce smoothly the amplitude of the signal closer to the edges of the extreme point signal, which becomes zero or close to zero. In addition to reducing artifacts due to the exceeding of limit values, various window functions were developed for suppression of the artifacts. These are the so-called "side lobes" or imaginary frequency components of the spectrogram. This artifact occurs near the "main lobes" of the true frequency components

(so-called "spectral leakage" - Lyon [13]). Different window software functions have different sensitivity (dynamic range) which determines their ability to suppress the side lobes.

For the maximum amplitude (about 0.16 V), which was fixed during the entire period of recordings on the selected range of frequencies (0.01 to 0.3 Hz), the dynamic range of the signal is 38.3 dB. So in the “window function” settings we used the setting of "Hamming" or close to this the "Cosine-Bell" setting.

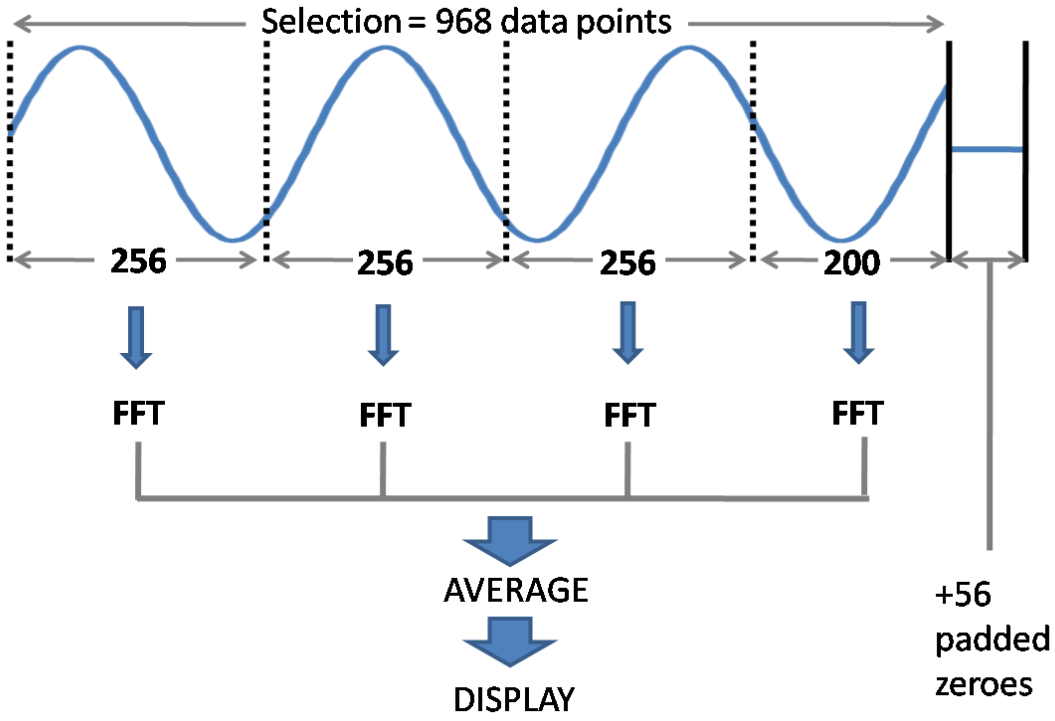
In these settings the level of suppression of side lobes is >40 dB [14]. This method ensures that stronger lateral petals do not mask the possible shortcomings of the spectral components. The side effect of the use of window software functions is some extension of the "central lobe" of the true frequency. Visually it displays as an increase in the width of the amplitude peaks on the spectrogram. Different window functions cause such effects, as described above. The "central lobe" of the spectrogram expands nearly 2 times in comparison with the spectrogram itself without the use of windowing functions. These functions are not critical for the selected frequency resolution. The properties of various window software functions can be found in detail in the work of Harris [15].

Thus, when choosing a particular window function, the researcher also faces the challenge of finding a compromise between the degree of suppression of artifacts ("side lobes") and the concomitant effect of the expansion of the "central lobe" (the real spectral component). This may hinder the differentiation between closely spaced frequency peaks. It should be noted that the various window functions attenuate the signal amplitude to

varying degrees. So "Hamming" function changes the amplitude to – 5.5 dB compared to the original signal [14], which corresponds to a decrease in the amplitude of about 53%.

The last parameter that can significantly affect the results of spectral analysis is the degree of overlap of the FFT blocks. One can carry out the analysis without overlap or there may be overlap to varying degrees: 25%, 50%, and other values. If you select no overlap the analyzed fragment will be divided into several parts that are multiples of the chosen number of FFT blocks. If necessary a series of zeroes can be added at the end of the signal array for a whole number of such parts. In the next analysis step an individual spectra will be calculated for each part, and the resulting fragments averaged to obtain the spectra of whole fragment (Fig. 2). For example, if you select the overlap of 50% then the analyzed fragment will be divided into a larger number of parts. These parts are also a multiple of the selected number of FFT, so these parts overlap each other by 50%. Similarly, for each part of the spectrum that is calculated, the following analysis results were also averaged. This gives a more accurate result, especially with applied window software functions, so in most cases it is recommended to apply the overlap.

Fig-2. Scheme of the algorithm for constructing spectrum in the software program Chart 5 (according to Chart Software Manual [16]) without overlapping of the fragments, with the addition of a series of zeros at the end of an array of points, and the number of points multiple by the FFT size = 256.



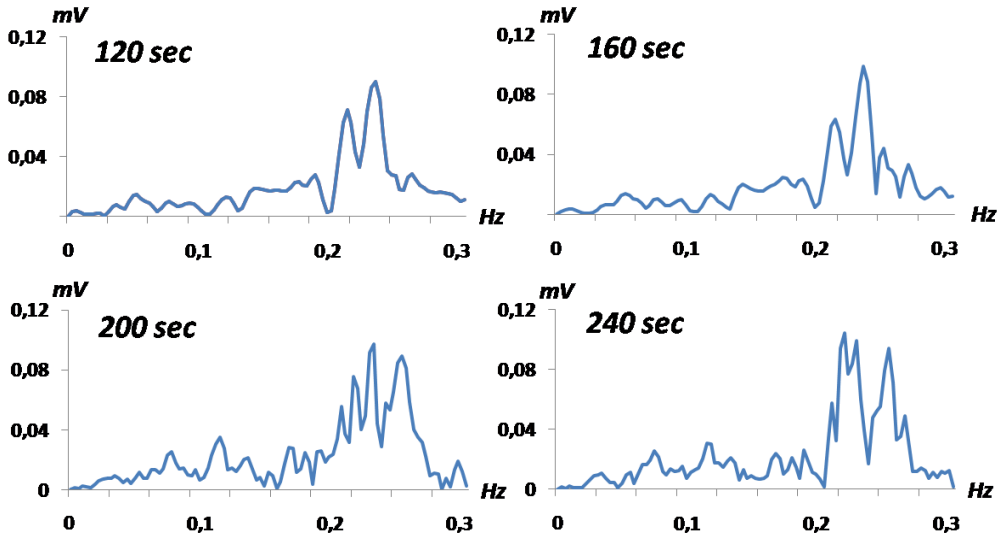
In addition to these three parameters, there is an option that removes artifacts from a signal component at a frequency of 0 Hz (the DC component). In most cases it is recommended to apply this option, because the DC component usually occurs as the DC offset, and has nothing to do with the investigated signal. Finally, it is important to remember that the maximum frequency (so called Nyqist frequency), can be reproduced in the spectrogram, according to the limit described in Kotelnikov theorem [17]. This must be strictly less than F_d / 2. Therefore, if you want to explore the spectra at frequencies up to 500 Hz, the sampling rate of the signal should be greater than 1 kHz. In our study, when F_d = 100 Hz, the spectrum can be adequately displayed only with frequency components less than 50 Hz. The SVFs are most pronounced in the low frequency range of 0.01-0.3 Hz, which are significantly below this limit.

5. The Influence of Time Recordings to the Accuracy of Mapping Components of the Spectrum

The accuracy of the representation of the spectral components depends on the duration of the analyzed fragment. The larger the fragment time duration, the narrower the 'lobes' of the spectrum. At infinite duration, the width of the petal tends to appear as a single line. Obviously, for adequate display of the slow-wave spectral component the length of the source fragment should be as big as possible. In our study the minimum frequency of interest was 0.01 Hz. This corresponds to an oscillation period of 100 seconds. Therefore, with the length of an analyzed fragment of at least 100 seconds it is possible to detect one period on this frequency. Previously, we had chosen empirically the optimal duration of the Mf-REG recordings, which should be at least 180 – 200 seconds. Fig.3 clearly demonstrates differences in the spectra for fragments of different lengths (the other parameters were unchanged with more details in the figure 3 caption). Increasing the length of the analyzed fragment leads to a narrowing of the peak's width and a slight increase in the amplitude of the peaks. Broad plateau-like peaks are split into more narrow and pronounced

peaks. For practical purposes, it is reasonable to choose an analyzed fragment with extra duration. In this study we chose 240 seconds, which allows the capture of more than two recording periods at a frequency of 0.01 Hz.

Fig-3. Resulting spectrogram in the range of 0.01 - 0.3 Hz at different durations of the analyzed fragment, all other parameters are the same.



Spectral analysis is an invaluable tool for studies of periodic oscillations of different natures. Analysis procedures can be implemented in many available mathematical and statistical software packages, both commercial and freely redistributable. The settings outlined above are basic, and are usually present in such programs. The optimal settings, as well as the duration of the analyzed fragment of the original signal, are selected empirically. However, their choice is important in the context of the goals and objectives of a particular study.

6. The Results of the Analysis of the Svfs

Visual comparison of average spectra of the SVFs in both hemispheres of the three subject age groups showed some differences (Fig.4). The younger age group were characterized by the maximal amplitude values of peaks in the range of 0.09 and 0.12 Hz for both left and right hemispheres. Alternatively, both hemispheres of the subject group of middle-aged people are characterized by "flat" spectra in all the investigated frequency ranges. There is no clear maximum value. It can be noted that two small groups of peaks, the first of which roughly corresponds to the frequency of the younger aged group and the second middle-aged subject group, are within the frequencies of 0.2-0.24 Hz. For the group of ageing (aged) subjects we observed in both hemispheres a group of peaks in the range of 0.14-0.18 Hz.

Fig-4. Averaged spectra of the amplitude of the SVF for the left (L) and right (R) hemispheres for patients of three subject

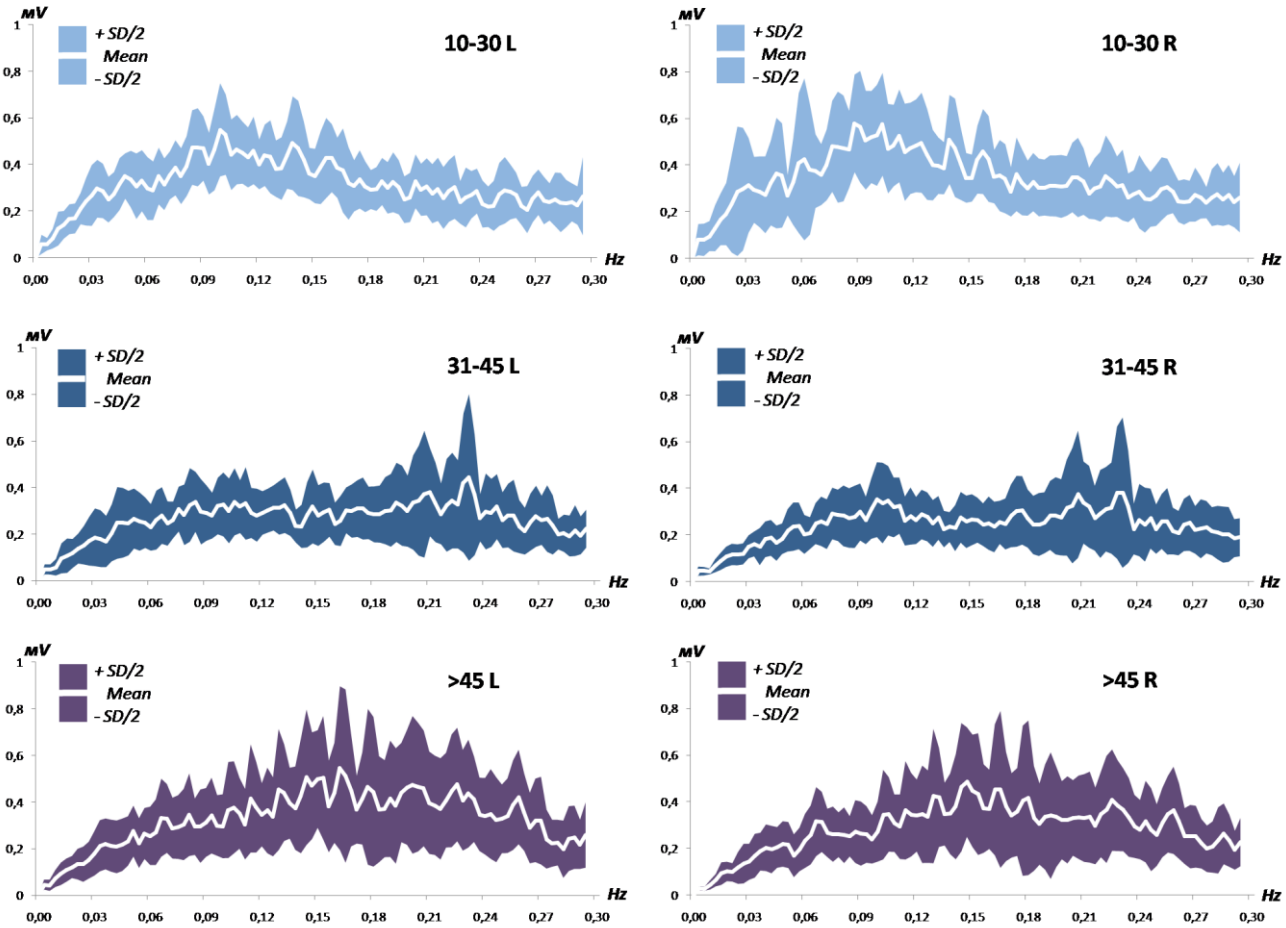
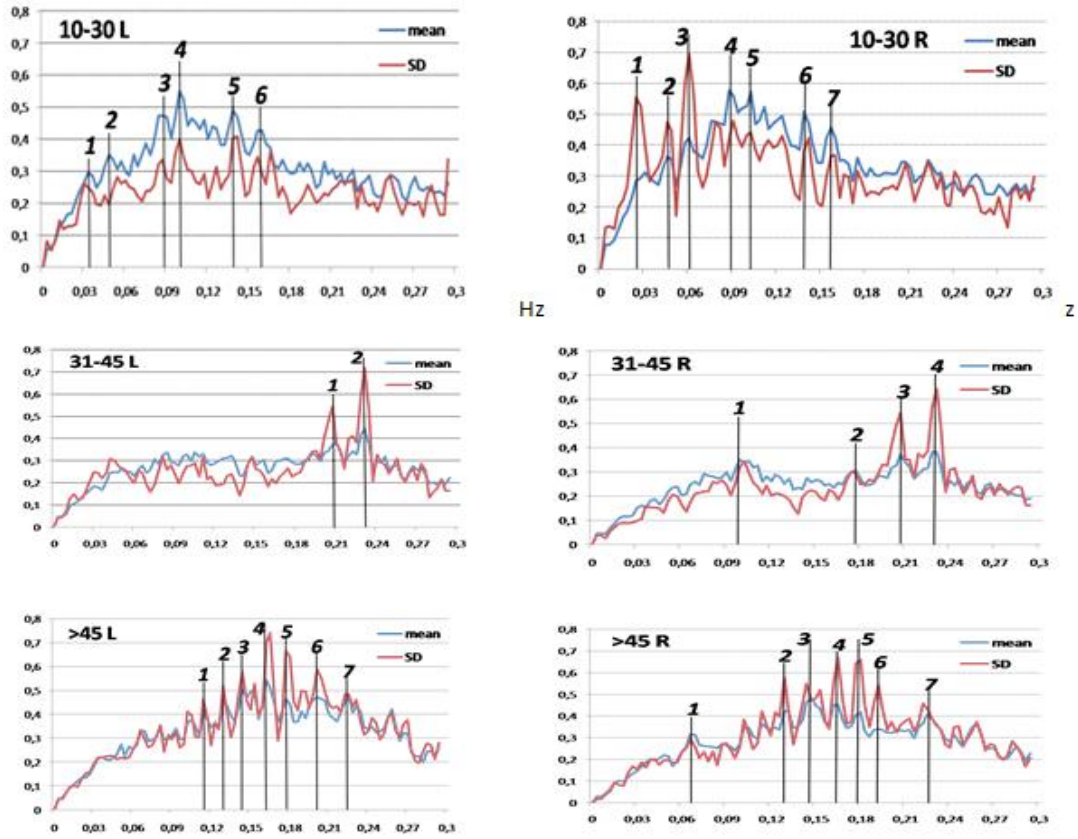


Fig-5a. Groups (10 to 30, 31-45 and > 45 years) in the tested range of 0,01-0,3 Hz. For each group the average (white line) and standard deviation (grey region) is displayed.



This figure shows that SD in different regions of the spectrogram have a different ratio of SD and mean value. This does not explain that this measurement of '0' excludes the possibility of error and that these results do not encompass a sufficient number of subject measurements. However, these analyses may represent a new, previously unmeasured phenomena of cranial fluid dynamics and brain physiology. The ratio changes with the position on the scale of the subject's spectrogram and depends on their age. Some preliminary correlations of SD and Mean in different regions of the spectrogram and at different ages here displayed in Fig.5a and 5b.

Fig-5b. Graphic (a) and table (b) representation of ratio SD/m for different frequencies in the age groups: young (10-30 years), middle-aged (31-45 years) and ageing people (>45 years). All people are physiologically and neurologically normal. Blue and red curves depict the mean value and SD respectively.

Age Group	Peak #	Freq, Hz	Mean, mV	SD, % Mean
10-30 Left	1	0,033	0,30	83
	2	0,048	0,35	55
	3	0,087	0,47	71
	4	0,099	0,55	73
	5	0,138	0,49	82
	6	0,156	0,43	81
31-45 Left	1	0,21	0,38	100
	2	0,231	0,44	162
> 45 Left	1	0,114	0,42	112
	2	0,144	0,51	115
	3	0,162	0,54	129
	4	0,177	0,47	144
	5	0,201	0,47	125
	6	0,225	0,48	103
10-30 Right	1	0,03	0,31	131
	2	0,045	0,36	130
	3	0,06	0,42	164
	4	0,087	0,58	72
	5	0,102	0,57	77
	6	0,138	0,51	75
	7	0,156	0,46	80
31-45 Right	1	0,099	0,35	91
	2	0,177	0,31	96
	3	0,207	0,37	145
	4	0,228	0,38	148
> 45 Right	1	0,066	0,32	94
	2	0,129	0,43	136
	3	0,147	0,48	97
	4	0,165	0,45	148
	5	0,18	0,42	159
	6	0,192	0,34	159
	7	0,225	0,41	107

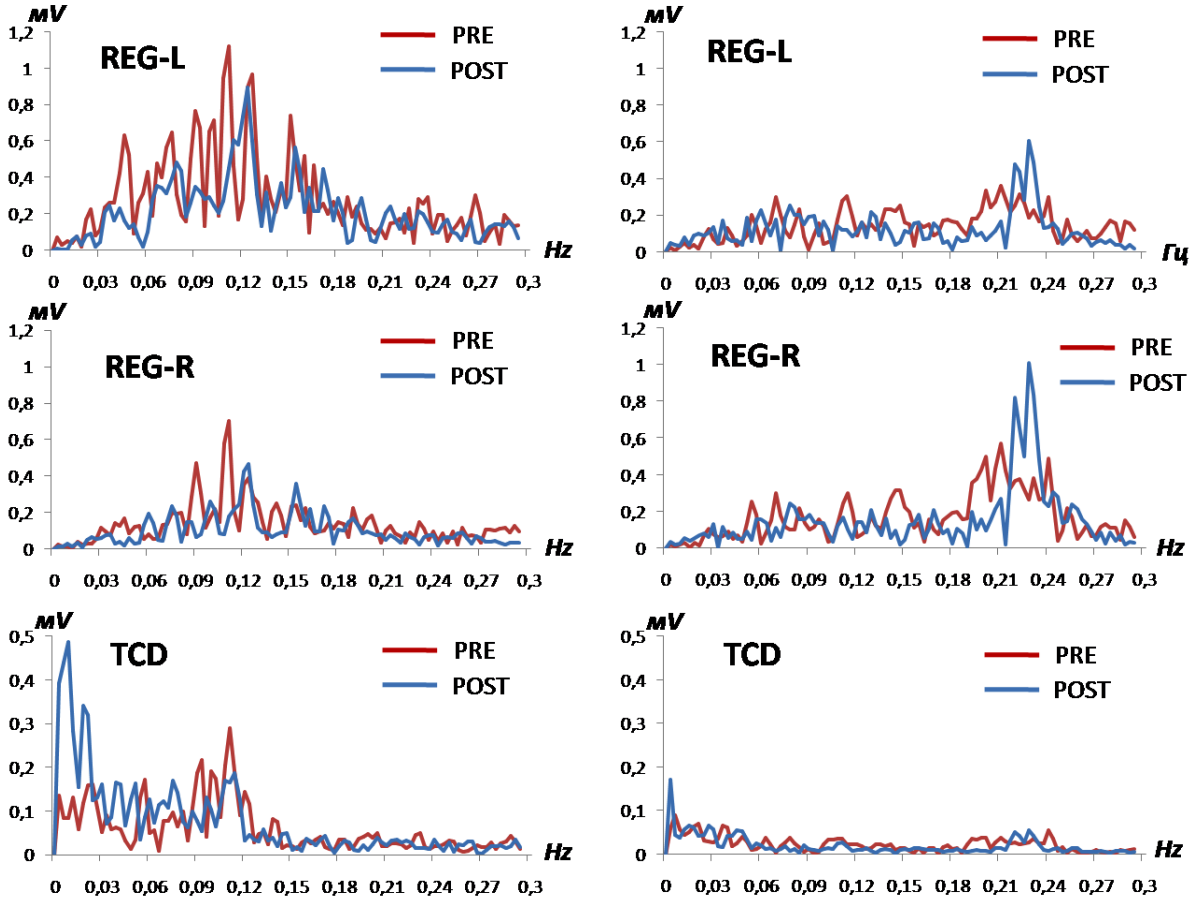
It is significant that in the recording segment with the middle-aged subjects there is a comparatively small number of peaks, and that these amplitude peaks are more pronounced for the other age groups. This corresponds with previous "Prognosis" investigations [10]. However, the physiological mechanisms may be different: they may be simply circulatory, but may be accompanied by a secondary auto-regulatory nerve control mechanism cannot be excluded. Changes of sectors at the spectrum diagram junction with the middle aged subjects are consistent in displaying both a low and high ratio of SD to mean value. We believe that these changes may have informational significance for diagnostic purposes, and will be a subject for further investigations. One hypothesis is that in the different fragments of the SVFs there are different physiological mechanisms or different components taking place in the process of autoregulation. If this is so one can conclude significant practical applications of this physiological phenomena, but the role of CSF is impossible to exclude. A comparison of individual spectra within these age groups showed a difference between hemispheres only for the middle aged group – their left hemisphere showed a series of peaks at frequencies of 0.1-0.15 Hz, but their right hemisphere peaks are evenly distributed. Accurate quantification of the observed differences is difficult because of the high variability of individual spectra in all groups (SD not less than 30% of the mean). Unfortunately, with such high variability of the individual spectra of the SVFs, the volume of investigated samples (30-45 subjects per group) is clearly not sufficient for quantitative

comparisons and conclusions about the statistical significance to support the visually observed differences. We hope to reach a statistically significant level of difference by increasing the number of subjects in each group in the course of further research. These study results also indicate that we need to revise the subject age groups to make further studies more effective statistically.

7. Examples of Changes in Individual Spectra of Mf-REG and TCD Recordings Before and After Osteopathic Manual Treatment

For the evaluation of the efficacy of osteopathic treatment Mf-REG and TCD recordings were carried out both before and after these treatments. Two patients with approximately the same clinical status and symptoms indicated different neurological insufficiency. One patient demonstrated a moderate disturbance of their afferent/efferent nervous system, but the second patient had pathology localized mainly in their central nervous system. The first patient (E.Z. female, 27 years old) had psycho-somatic disorders not associated with the condition of her vascular system. The second patient (G.B. female, 75 years old) had a clear disturbance of the vascular system of her brain associated with changes in blood volume and CSF. The treatment selected was the osteopathic technique called 'drainage of the venous sinuses', because it encompasses a global effect on the different components within the cranial cavity and aims to support the auto-regulatory circulatory-metabolic maintenance of brain activity. It can be seen that the number of pronounced peaks in the Mf-REG spectra for the left and right hemispheres of both subject patients was decreased after treatment (Fig.5). The first subject's spectrum demonstrated a main amplitude peak at a frequency of around 0.12 Hz after treatment. The second subject's spectrum was characterized by double amplitude peaks at a frequency of 0.22-0.24 Hz. The TCD spectra of both subjects demonstrated maximal peaks at the lower boundary of the investigated frequency range of 0.01 to 0.02 Hz. However, it could also be a manifestation of the artifacts of spectral analysis.

Fig-6. Individual SVFs spectra before and after osteopathic treatment, recordings include REG left (REG-L) and right (REG-R) hemispheres and individual TCD for two female patients with slight neural disorders (diagnosis described above in the text).



If the changes observed in the spectra of both hemispheres of REG and TCD are the result of treatment effects, it can be assumed that it has a "synchronizing effect". Instead of many small peaks in the amplitude before the treatment, more pronounced peaks at certain frequencies and the total number of peaks were reduced. Differences before and after treatment for the second subject were much more pronounced. This means that in the second case, the therapeutic effect of the osteopathic technique is more pronounced, which is consistent with the subject's presenting condition. Indeed, if in the first case, the pathophysiological processes do not affect the integration of the systems that are responsible for blood flow within the cranium, with the second case there is known pathology

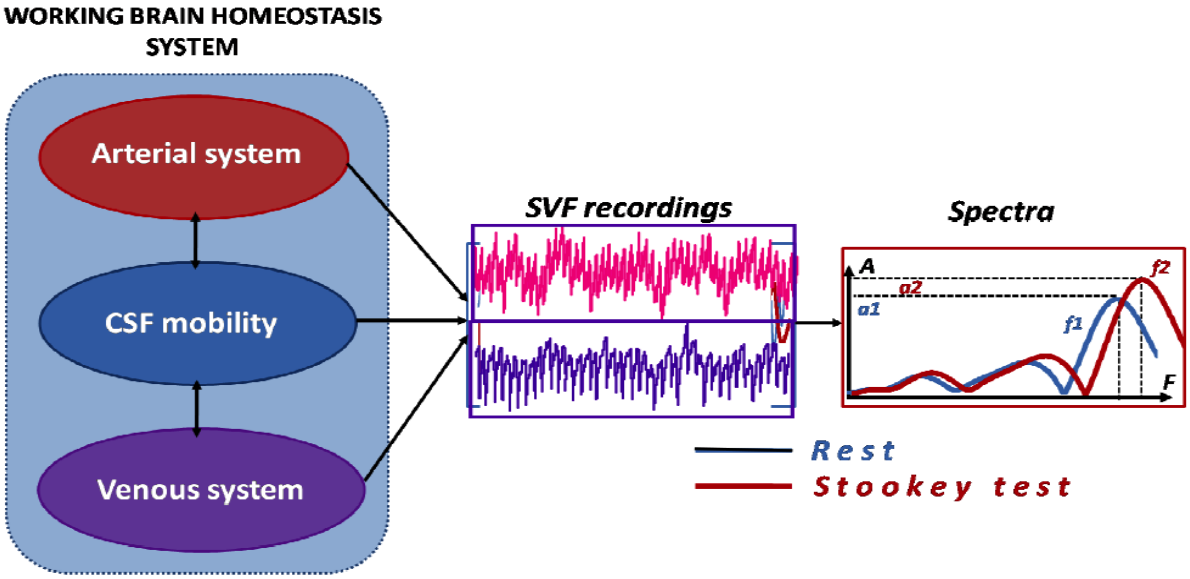
directly connected with the subject's physiology. However, it is necessary to take into account that any response to osteopathic treatment may depend on gender and age. In addition, TCD spectra and especially Mf-REG have a high variation with different subjects. Thus these visually observed changes need further statistical support which could be achieved with an increase in the subject group size. Spectral analyses may help to understand the real difference between subjects of different ages and gender. It is possible to conclude from this study that significant effects occur in the cerebrovascular, CSF and cranial bone systems post osteopathic treatment, and that osteopathic manual diagnosis may be of use as a functional test for differential diagnosis in altered or dysfunctional cranial fluid dynamics.

We may assume that the SVFs are the result of the integrative action of cranial fluid mechanisms that influence the circulatory-metabolic maintenance of brain tissue and subsequent brain activity. Apparently, their systemic parameters depend on the interactions determining the homeostasis of the working brain, and therefore reflect both the disruption of the normal physiological functioning of individual cranial systems and their components. These study results indicate that in conditions of physiological normality the SVFs group averages are highly variable, making the Mf-REG/TCD analyses less informative with a subject group size of 30 persons or less. However, in general it can be noted the presence of right-left hemispheric asymmetry in the young age group, and some differences between the other age groups. However, even minor neurological pathology was found to affect the dynamics of the SVFs' measurements demonstrated by changes in the number of spectral peaks and different amplitudes in their individual spectra.

It is important to mention that the data did not allow conclusions with confidence about the age and hemispheric differences of the SVFs due to the high individual variability of the individual analyzed measurements. When new additional data is collected and/or more detailed age and gender classifications applied, the differences may be clearer statistically. The subject's medical history and independent diagnosis of their physiological and neurological conditions will enable the use of functional physiological perturbations such as breathing perturbations to influence the physiological status of each subject. We believe that SVFs can provide important objective measurements related directly to a person's cerebrovascular fluid dynamics. Spectral analysis of SVFs as indicators of cranial fluid volume dysfunction has the potential to refine neurological diagnoses by comparing normal circulatory-metabolic rates of change in blood and CSF (99% water) volumes, and their effects on the circulatory-metabolism of the brain and neural activity post trauma or with neurodegeneration.

8. Conclusion

Fig-7. Schematic illustrating the application of spectral analysis for studying the interaction of the cranial physiological systems.



The data collections and analyses presented in this paper have indicated the possibilities of spectral analysis in physiological studies. This may open the way for new methodology for the study and
monitoring of physiological functions by analysis of waveform fluctuations, which represent integration of simple periodic processes which functionally interact to achieve overall brain homeostasis, which is necessary for the survival of the human organism as a whole. Spectral analysis of slow fluctuations, reflecting the interactions between the venous, arterial and CSF fluids may provide an index for quantitative evaluation of these physiological interactions.

This study concludes that SVFs reflect some of the complicated functioning of the brain's physiological system while dynamically engaged in its integrative interactions, which are focused on the functional goal of survival.

Unfortunately, the collected data didn't provide all the significant information concerning these interactive systems, composed of a number of physiological structural components, which supports brain homeostasis.

This data does demonstrate one possible way to study the complicated intracranial physiological system by means of spectral analysis. A schematic representation of such an approach is shown in fig.7, which illustrates the principle of the study of functional intracranial relationships, based on an evaluation of spectrogram patterns.

Finally, it is important to note that our studies focused on the subject's reaction when standing with closed eyes on a "stabiloplatform" which measures postural deviations of the body from the absolute vertical position. There was no measurable correlation between SVFs and the body's oscillations or postural control. This contradicts the hypothesis of a central body "pacemaker" potentially generating co-ordinated periodic processes in the human organism to maintain equilibrium.

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