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Effectiveness of Arc synaptic adaptation therapy in the treatment of patients with tinnitus lasting for two years.

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Abstract

The nervous system may be subjected to natural deprivation and in spite of the fact that it is the decrease of the volume and number of nervous cells in relation to gradual sensory deprivation it is still the key process which serves for the optimization of the energy resources to the factual stimulation. However, for the scientists deprivation is related with something wrong, but it is a natural condition for human body in which the unused elements (too large population of neurons in relation to, for instance muscle mass) are reduced pro rata the reduction of stimulation level. Keeping the tissue, muscle structure or nervous system in excess alive when they are not needed would involve the necessity to keep alive certain structures (oxygen, blood, nutritional values) which are not used. That is why for instance, muscle mass is reduced in the absence of physical activity, the body notices it does not require the developed muscle structure which requires a lot of energy to maintain and decreases it. A similar process takes place in the sensory and nervous systems if the sight gets weaker due to gradual deprivation also the number of neuron units analysing stimulation as the electric potential gets significantly lower (Mark F, Howard Colman 2012). Based on the research of the motoric cortex deprivation in athletes affected with ALS (Amyotrophic lateral sclerosis) it may be noticed that together with the drop of the muscle mass also the motoric units of the nervous system undergo a significant

deprivation as a reply to decreasing electric potential coming from smaller muscle mass (TDP-43 Proteinopathy and Motor Neuron Disease in Chronic Traumatic Encephalopathy – Ann. C McKee). Gradual deprivation is also known when auditory pathway is concerned. Auditory pathway may undergo gradual deprivation due to progressing health problems of the patient (diabetes, circulatory problems), however the response of our nervous system to such gradual and consistent lowering of the stimulation level is the same as in case of the motor cortex. The number of neurons which serves the outlet of the auditory pathway (cochlea), as well as the mass of synapses which drop proportionately to decreased, spontaneously released activity of synapses are limited. As may be noticed, the nervous systems treats deprivation as the response to the change of the level and intensity of stimuli environment and adjusts the capacity of the nervous system to these changes. There already exists a large number of research indicating that the deprivation process of the central nervous system change due to decreasing level of stimulation from the peripheral sensors inputs with the sudden loss of the stimulation level is different. Sudden loss of sight (CBS; Lerario, Ciammola, Poletti, Girotti, & Silani, 2013) leads to hallucinations as the symptom of neurons excitation in the visual nervous system, sudden loss of the contact with a limb leads to chronic pain (Phantom Pain) and sudden loss of hearing leads to tinnitus (Pitskel, Merabet, Kauffmann 2007), (Lou et all, 2011).

Introduction

Naturally, what may obstruct the process of deprivation of the nervous system and its adjustment to decreasing stimulation in relation to the derivating peripheries is too quick drop of the activity of peripheral sensory parts activity. As the research show (Pitskel,Merabet,Kauffman 2007) a sudden deprivation of many centres leads to hyper-excitement of the whole nervous system, whereas the gradual deprivation increases the excitement for a few days and then gets decreased. Too quick process of deprivation which may relate to auditory or visual pathways generates negative symptom of too much developed nervous system in relation to quickly undergoing atrophy of stimulation coming from sensory inputs. The nervous system needs time for the changes taking place during the gradual deprivation; in case of a sudden cut-off of stimulation there is no time. The nervous system is not able to even start the process of decreasing its own structure. Thus, if the external auditory hair cells die within a second, the nervous system would not decrease the number of neurons and connections created thereby for example in the period of 10 years in such a short time; sudden cutting off of stimulation in case of auditory pathway is connected with a violent drop of glutamate, main neurotransmitter of the auditory nerve, and, consequently, the increase of excitement and spontaneous activity of synapses cochlear nuclei (2014 Calvin Wu, David T. Martel, and Susan E. Shore). The generated potential is a kind of information for higher neurons of the auditory pathway in high frequency range, where in silence there may occur a sound which is a danger. That is why even the activity of the Amygdala (S.J. Brooks 2011) which by the stimulation by silence also increases the activity of other centres in the brain generating, at the same time, a sudden drop of serotonin, tiredness and depression. The nervous system which is stimulated by silent surrounding all the time reads the sudden loss of hearing as life threatening condition and triggers special alert reaction of sympathetic system (life threat) and therefrom come sleep disorders in the patients suffering from tinnitus. Consequently, it leads also to plasticity disorder through decreased production of BDNF neurotropic protein and HT-5 serotonin. Generating of hyper-excitement of the auditory nervous system is called in modern nomenclature the tinnitus. Similarly as in the case of visual pathway, when the symptom of a sudden loss of stimulation is generating by the visual pathway (visual cortex) the picture hallucination, in the auditory pathway there occurs tinnitus as the impression of the sound in the form of a potential of spontaneous activity generated by too strongly excited synapses which send to neurons the information on the lack of stimulation from the auditory nerve. Because the auditory cortex is tuned to the receipt of different frequencies each synapsis and neuron have completely different frequency of release of spontaneous activity and neutral oscillation. This is manifested by generating of a sound characteristic for the patient, which depends on the deepness of the hearing loss (the number of synapses in dB level which lack the stimulation) and the pitch of sound (in what band a sudden loss of hearing occurred). Nowadays, there are different pharmacological or therapeutic technologies, however the research show that the nervous system under the influence of time after such sudden loss of hearing changes its structure. Hyper-excitement of synapses (tinnitus) takes a completely different generating manner. What is important, the changes relating to innervation take place already in the first days from the sudden loss of hearing and tinnitus occurring (Lukas Rüttiger 2013). After 24 hours from the sudden loss of hearing, the inner hair cells which lost the outher hair cells lose 30 % of auditory nerve fibers and ribbon synapses innervation. A different treatment strategy, adjusted to a certain case and the characteristics of the changes should be applied along with the occurring changes. Pursuant to the research (Dave R.M Langers,Emile de Kleine 2012) it may be claimed that in the first years the tinnitus takes the form of spontaneous activity Schaette, R., and Kempter, R. 2006), which means that the nervous system is strongly excited but still preserves its structure neurone-cell, and in the longer period of time the innervation gets weaker and the nervous system migrates the neurones from the populations affected with hearing loss for the sake of well hearing neurones.In subsequent years, larges changes occur within the nerve system.A different therapy should be selected for each of these stages. Pursuant to the research (Chung H.K, Tsai C.H, Lin Y.C, Chen J.M 2013) it is known that rTMS may be an effective technology of treating the model in later years. However, apart for the tinnitus generators, which do not treat but silence the tinnitus there is no other technology. There is not therapeutic strategy for the patients which are affected with the first model of tinnitus (Chung H.K, Tsai C.H, Lin Y.C, Chen J.M 2013) of spontaneous activity. If the tinnitus is generated in the first stage as the symptom of lack of adaptation of the nervous system, and, by the same, of lack of decreasing the neurones number in relation to slow and gradually proceeding, decreasing stimulation the solution and therapeutic purpose in generated tinnitus of this type should be a therapeutic strategy with the aim of decreasing the neurones up to the level of the exsting loss of hearing.The only manner is the possibility of mapping of the gradual deprivation of hearing in already existing sudden loss of auditory cells. With the help of the innovative technology, as the first in the world, with the use of decreasing accoustic stimulation our aim is to decrease in time the number of neurones in the whole auditory pathway and mapp the gradual proceeding loss of hearing, the effect of which would be the reduction of hyper-excitement of the patients nervous system (tinnitus). Our goal in the present clinical trial is also to check the reaction of the nervous system, which was not adapted during the sudden loss of hearing, the symptom of which is the tinnitus to the stimulation, again decreasing in time. The inner cell would be excited by the signal which is to map the gradual deprivation in intensity (dB) and time (t), as well as frequency. If it would be possible to adapt nervous system and the synapses mass to the already existing sudden loss of hearing by mapping of the gradual deprivation process we would be able to confirm the revolutionary technology, whose bases and possibilities of acting would be based on natural deprivation processes and the power of gradual decrease of the stimulation level in time. Bioacoustic sp.z.o.o Ltd. Company created the device called the synaptic stabilizator as well as therapeutic programme called Arc synaptic adaptation therapy, which aims at decreasing of the number of neurones up to present loss of hearing with the use of accoustic stimulation which gets lower in time, the same as in the case of gradual loss of hearing. According to the authors of the technology (Adam Pabiś et all,. 2014) the stimulation, decreasing in time should start a process similar to the one taking place during progressing loss of hearing the effect of which should be decreasing the population of neurones up the present sudden loss of hearing, which, in turn, should lead to the reduction of tinnitus and hyper-excitement. The purpose of our trial is to test the effectiveness and efficiency of this therapy in 25 patients with tinnitus lasting for no longer than 2 years.

Subjects

25 patients suffering from tinnitus lasting for no longer than two years were examined in terms of possibility to tinnitus creation due to sudden loss of hearing related to sudden death of external auditory hair cells in the inner ear or destruction of innervation of ribbon synapses or neurones of spiral ganglion. All persons are right-handed, none of the 25 persons experienced accoustic trauma within the last 10 years, none of them works in noisy environment. All persons reported tinnitus occurring during a day in a non-siginificant situation (no physical activity, the position of cervical vertebrae does not expos the strong muscle tension, no work at heights) or after waking up. Previous audiological tests did not indicate the loss of hearing within speech recognition from 125 Hz to 10 kHz.The persons taking part in the trial did not suffer from previous otitis media or other laryngological disorders. 10 persons out of 25 hear unilateral tinnitus on the right side, next 15 persons report bilateral tinnitus. When asked whether they hear the tinnitus more in the head than in the ear the

patients answered in agreement that in the ear, in the structure close to the ear. In TQ scale, in the range of the level of the tinnitus nuisance,10 persons reported the tinnitus as very troublesome, 9 persons in the TQ scale described tinnitus as annoying and 6 persons indicated the tinnitus in the TQ scale as less annoying. The 25 persons included in the trial are within the age group (25-45 years).

Control group (Plecibo)

6 subjects with bilateral tinnitus were subjected to the placebo effect in the same period. These persons received the Arc device with the same algorithm of frequencies as tested persons but the signal during the therapy was not changed within weeks and months. The signal was the same; it did not get lower in frequency and intensity.

Qualification

- 25 patients qualified for the trial do not suffer from hearing loss within the speech perception (125 Hz to 10 kHz) and the loss of hearing in the higher frequencies above 10 kHz to 20 kHz.

- 6 patients with bilateral tinnitus were subjected to the placebo effect with non-stimulation change in time, frequency of signal and threshold level decrease.

- The tinnitus in none of the patients lasts for longer than 2 years.

- In the history, none of the patients reported additional symptoms accompanying the tinnitus, i.e. auditory hypersensitivity in any scope, the tests of ABR trening –UCL were carried out which confirms that the patient is not affected with the model of enhanced synchronization pursuant to tests ( ) but is affected with the model of spontaneous activity ( ).

- The detected sudden loss of hearing is confirmed with the measurement of TT (Tinnitus Tone) TFR (Tinnitus Frequency Reduction), which means that after giving the tone in the range the sudden loss of hearing which caused the tinnitus is suspected it gets silent for several seconds under the influence of short-term accoustic stimulation in the band of sudden loss of hearing, whereas in other bands such effect did not occur.

- The patient's results of plasma serotonin or blood serum in plasma is correct - HT-5 there are no sleep disorders, and, by the same, due to the high level of BDNF the plasticity of the central nervous system is high and correct, without any visible disorders in the form of depression, anxiety conditions, lowered level of sleep quality. That is why we may foresee high influence of stimulation during the carried out test which is not distorted by the factors which could distort or significantly decrease the results, taking into account the significance of the BDNF neurotropic proteins and serotonin levels in foreseeing the effects of treatment (Owen M. Wolkowitz, Jessica Wolf 2011).

All audiology tests were carried out with the use of audiology equipment

a) Labat Company high frequency audiometer - AUDIOLAB AUL 14 002 5Vdc - Assorbimento -.5 A in the Centre of Modern Audiology Kinetic.
b) ABR/ASSR Trening - Labat- EPIC - PLUS EN ISO 389 .
c) EEG tests -EMOTIV EPOC + 14 channel wireless EEG
d) Serotonin testing HT-5 serum / blood plasma. ALAB sp z.o.o Ltd. Co./ The Nencki Institute of Experimental Biology

Serotonin testing – The Laboratoy of the Institute of Experimental Biology.

Through the qualification, each patient underwent a series of audiological and neurobiological tests. ABR trening (Qiang Song, Pei Shen, Xei Li 2013) to demonstrate whether the patient, apart from destroyed external cells has no other hidden sudden loss of hearing within the inner ear connected with the destruction of ribbon synapses or the neurones of the spiral ganglion. High frequency audiometry was carried out to verify the depth, widht and the manner in which the extrenal cells died in the band from 125 Hz to 20 kHz (audiogramme characteristcs). TT/TFR test (Tinnitus Tone Frequency Reduction) is based on the stimulation with the continuous tone in the band where the loss of hearing is suspected, and, by the same, by the enhanced activity of synapses in the cochlear nucleus.

The results of tests / Measurements and materials

Tinnitus Questionarry TQ Global – the tinnitus intensity in relation to time of the day (Pursuant to a modified questionnaire used in the international standards) .

TQ Global (Tinnitus Questionary) each of our patient had to indicate the range of time of the day when they heard the tinnitus quietest and when loudest.

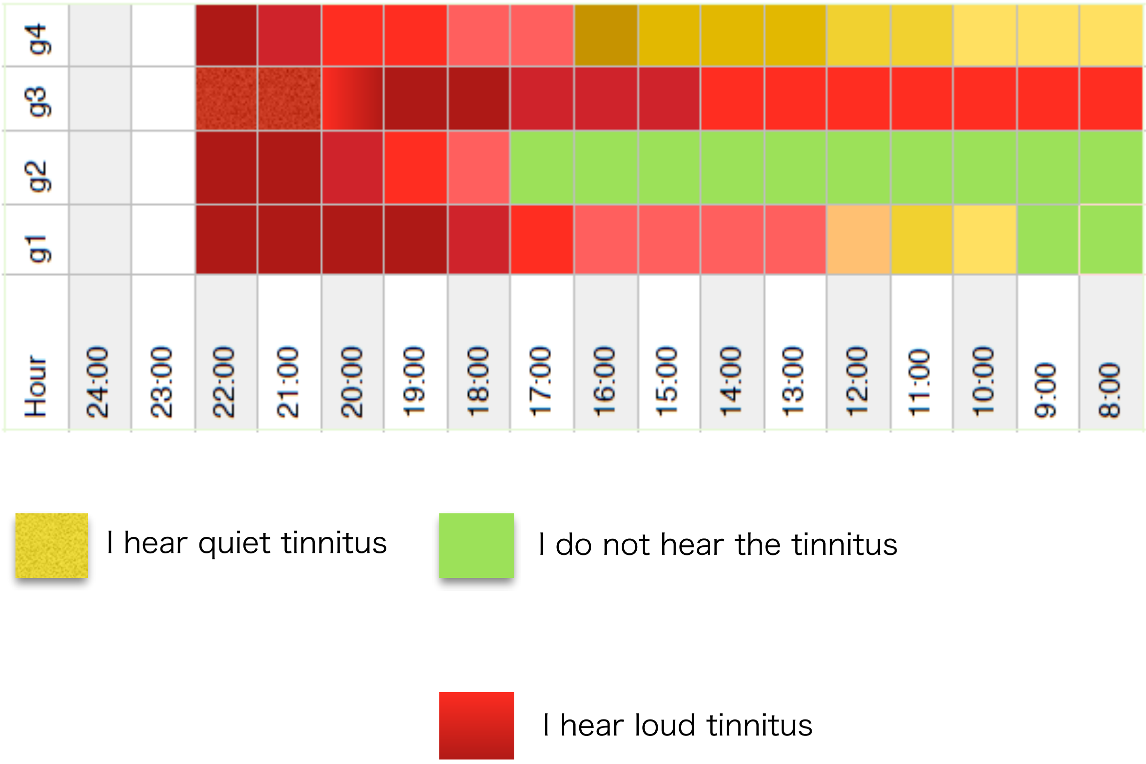

Table No. 1. depicts four groups (g1,g2,g3,g4) of 25 patients divided into those hearing the diferent level of tinnitus loudness depending on time of the day.Group g1 (5 persons) indicated in the TQ global questionnnaire that they do not hear the tinnitus in the morning, at noon it gets stronger and reaches the loudest value in the evening, the influence of the accoustic stimulation is moderate. Group g2 (8 persons) indicated that from the morning till late afternoon they practically do not hear the tinnitus it is not noticed by them, however in the evening the tinnitus perception is the loudest; what is interesting, the results of TT/TFR test was the best in this group which may prove that the sudden loss of hearing in this group had the least negative influence on the reduction of auditory nerves fibres and ribbon synapses. Group g3 (7 persons) did not notice any difference in the tinnitus loudness depending on time of the day, they hear the tinnitus.

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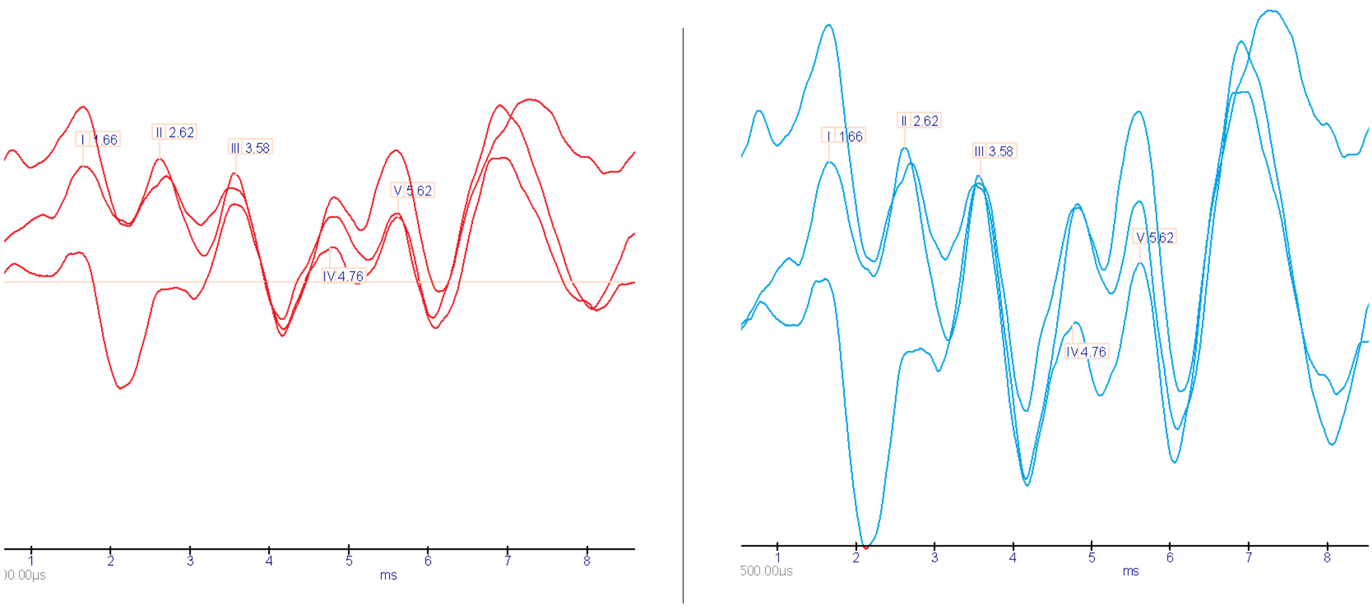
ABR training (Auditory Brainstem Response training protocol)

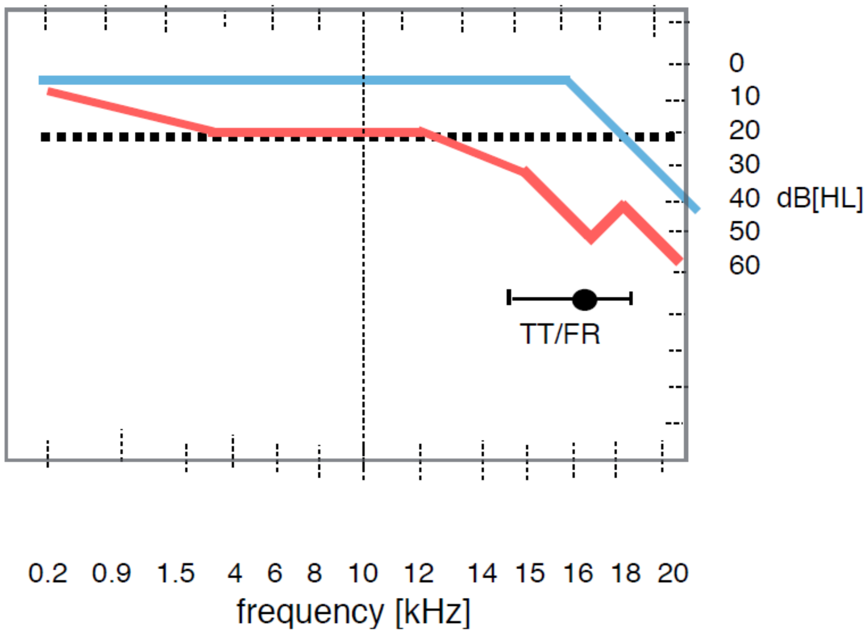
ABR was carried out in all patients to determine whether, apart from standard loss of hearing on high frequencies which was related to sudden death of external cells, in other sectors of the cochlea there occurred death of synapses or neurons of spiral ganglion under the external auditory cells. ABR test was carried out with the use of the equipment by Labat Epic-Plus in a standard configuration of electrodes with the use of high frequency Sennheiser (HDA 300) headphones. ABR training was carried out for the frequency from 4 to 10 kHz in the frequencies bordering the sudden loss of hearing. Type of accoustic impetus – crack 50 μs of 90 dB intensity, rate of signal providing 11 clicks/s (rate 11clicks/s). The result of the test in 10 persons with unilateral loss of hearing and tinnitus indicates the proper morphology and amplitude results for the healthy ear not inflicted with the loss of hearing and the increase of wave II amplitude and decrease of wave V amplitude which is characteristic for tinnitus. ABR result did not indicate any differences in the range not inflicted with the sudden loss of hearing. There was no chart characteristic for sudden loss of hearing connected with the destruction of synapses or neurons of spiral ganglion.



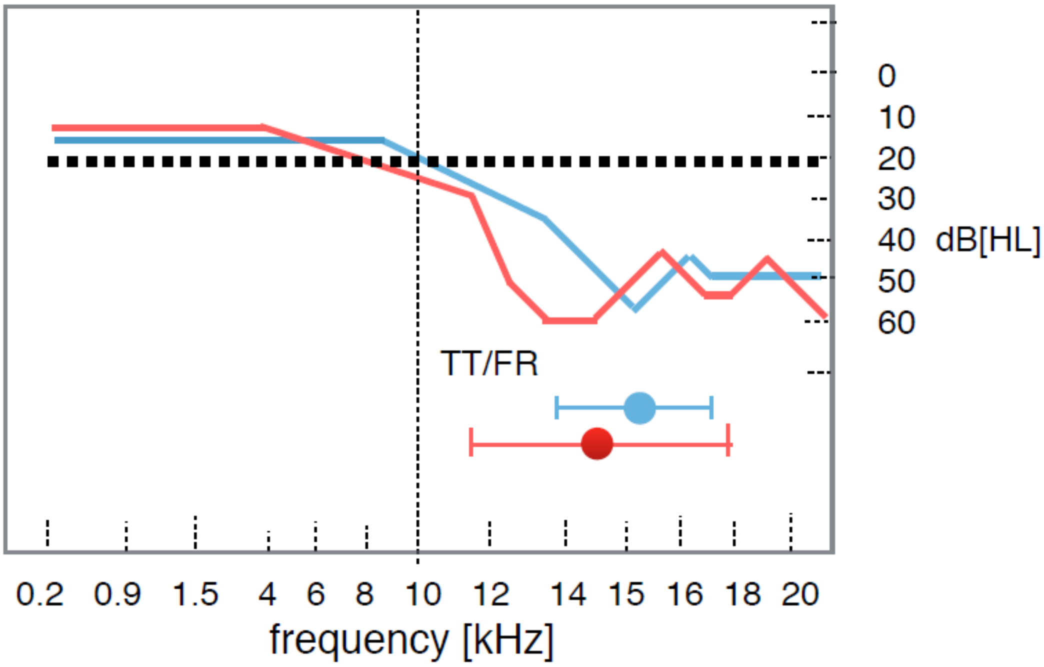
The chart No.1 above depicts the average morphology and amplitude of waves I II III IV V of ABR results (induced potentials of the brain stem) for right and left ear in patients with unilateral loss of hearing on the right side. The characteristic drop of amplitude for all waves on the right side where the sudden loss of hearing occured is visible but the chart itself did not reveal the destroyed ribbon synapses or the neurones of the spiral ganglion in frequencies from 4 to 10 kHz in this band.

The result of high frequency audiometry

In 10 persons with unilateral loss of hearing the range of the sudden loss of hearing was precisely visible. The presence of the loss in the range from 10 to 16 kHz on the right side did not occur on the left side, which additionally confirms the theory that the reason of the tinnitus is the sudden loss of hearing and lack of possibilities of the nervous system to adapt to this change (13.03.2013 Lukas Ruttiger - The Reduced Cochlear Output and the Failure to Adapt the Central Auditory Response Causes Tinnitus). Loss of hearing in the ear where the tinnitus was audible in the band from 10 to 16 kHz it was not noticed on the left side where, for most of the patients was the range of normal hearing. Above 16 kHz on the right and left side a gradual loss of hearing was noticed, which is characteristic for the age of the patients, however with greater intensity in the ear affected with sudden loss of hearing.



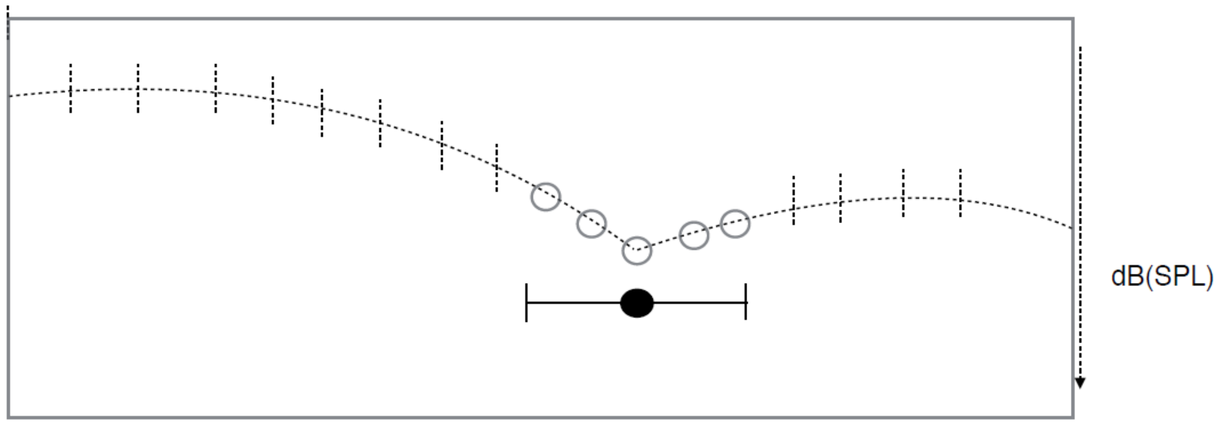
The above audiogramme depicts the average range of audibility of 10 patients with unilateral tinnitus. A difference between audibility for most frequencies between right and left ear is visible. The biggest differences start from 14 kHz and we decided to divide the loss of hearing referring to the loss of hearing on the left side into the part which died gradually with the age and into the other part which died sudden death due to sudden loss of hearing (visible loss of hearing on the right side invisible on the left side). From 14 to 18 kHz a maximum characteristic for a sudden loss of hearing is visible where, then, above 18 to 20 kHz a standard loss of hearing proceeding with the age is visible, similar curve is visible on the left side. Additionally, the parameters of tinnitus tone and frequency reduction (TT/FR) indicate when the patient did not hear the tinnitus after receiving pure tone of a certain frequency. From 14,5 to 18 kHz for most of the patients was the biggest influence of the stimulation on the tinnitus reduction, above 18 kHz to 20 kHz the patients reported they hear the tinnitus or whistling together with the tone given through the headphones.



The above audiogramme depicts the average range of audibility of 15 patients with bilateral tinnitus. The loss of hearing, both for right and left ear had its beginning in the high ranges of frequency above 10 kHz until 16 kHz. Maximum characteristics for sudden loss of hearing were visible between the frequencies from 12 to 17 kHz. Similarly as in the group with unilateral loss of hearing and tinnitus the range of the loss of hearing (width in frequencies) could be divided into range caused by sudden loss of hearing and the ones which were lost gradually in time (progressing loss of hearing) in the group of persons with bilateral loss of hearing. The average of TT and FR reaction (tinnitus tone and Frequency Reduction) divide the frequencies which died suddenly on the right and left side again. The TT and FR results coincide with maximums of sudden loss of hearing. Above the reaction for reduction frequencies other frequencies were described as gradual loss of hearing where the stimulation did not reduce the tinnitus but was only audible, irrespective the patient's tinnitus and given 14 kHz and we decided to divide the loss of hearing referring to to the loss of hearing on the left side into the part which died gradually with the age and into the other part which died sudden death due to sudden loss of hearing (Visible loss of hearing on the right side invisible on the left side). From 14 to 18 kHz a characteristic maximum for a sudden loss of hearing is visible where, then, above 18 to signal.

TT/TFR (tinnitus tone frequency reduction)

The test, developed by Bioacoustic sp.z.o.o (Poland) Ltd Co. uses previous results of high frequency audiometry and it consisted in providing the signal in the range of the patient's hearing from 125 Hz to 20 kHz in the form of a continuous tone. The tone which, irrespectively the tinnitus characteristics silenced it not matter whether it was complaint was given from the lowest to the highest frequencies. Then the patient selected whether after providing of the tone of a given frequency (f) they still hear the tinnitus or whether they continue to hear mainly the sound from the headphones and do not hear the tinnitus or whether they hear both sounds. Thanks to this test the cells and the nervous system may be divided into the one affected by a sudden loss of hearing and the tinnitus and the progressing loss of hearing which was present before sudden loss of hearing occurred. The relevance of the frequencies which reduced the tinnitus was recorded as the period during which the patient, after being given the signal would not hear the tinnitus. The longer period of tinnitus silencing the bigger correlation between the present loss of hearing and generated tinnitus. In the ranges in which the shape of the audiogram indicated the possibility of sudden loss of hearing occurring the test confirmed that by a huge influence of the stimulation onto the tinnitus reduction.



f(kHz)	0.5 - 2	2-4	4-6	8-9	10 kHz	**12-13**	**13-14**	**14-16**	17-18	18-19	19-20
RT	T/S	T/S	T/S	T/S	T/s	T	T	T	T/s	T/S	T/S
t (TR)	0 sek	0 sek	0 sek	0 sek	1 sek	9 sek	60 sek	20 sek	1 sek	0 sek	0 sek
ZP											

The chart No. 2 together with table No. 2 indicate the factor of the tinnitus reduction in relation to the given tone of a certain frequency. RT – means the type of reaction (Reduction Type) whether the patient heard only the sound from the headphones or whether he heard both the tone and the tinnitus while being given a tone of a certain frequency. We nominated Tinnitus Reduction Time with the symbolem t(TR), i.e. for how long from being given the tone and turning it off the patient did not hear the tinnitus, the unit of this parameter is the time counted in seconds. ZP parameter means the compliance of repetitions of patient's reaction (how many times the given signal generated the same effect). Pursuant to the TT/TFR factor a stimulation protocol was prepared for all patients.

Testing of HT-5 serotonin in serum or blood plasma

The patient who was to be qualified for the trial of the effects of Arc synaptic adaptation therapy had to obtain a determined level of HT-5 serotonin in blood serum or plasma. In many fields of the science it is possible to foresee the effects of the therapy onto a patient through the level of BDNF protein level and strong correlation of HT-5 serotonin with the level of neurotropic proteins. It is known already for some time that BDNF protein and serotonin and their certain level provide the information about the mental condition of the patient but also the information on the plasticity of the nervous system onto the type of stimulation (the stimulation would have a positive impact or none). In our case we used this ratio for foreseeing the chances of the therapy influence, it is also obvious that it is not possible to evaluate the effects of the therapy which affects the nervous system knowing that its plasticity and possibilities of adapting to external environment equal to zero or are distorted (too low level of serotonin and neurotropic protein). Thus, we required our patients to have the highest level of BDNF proteins and serotonin ratio which was measured before beginning of the diet and physical activities which aimed at increasing all biomolecular ratios; the level was also measured after one month of physical activities and diet.

All patients but 3 obtained a significant result and the dietary and physical activity protocol was no longer required to equal the factors such as neurotropic protein and HT-5 serotonin. The tests were carried out in the Institute of Experimental Biology.

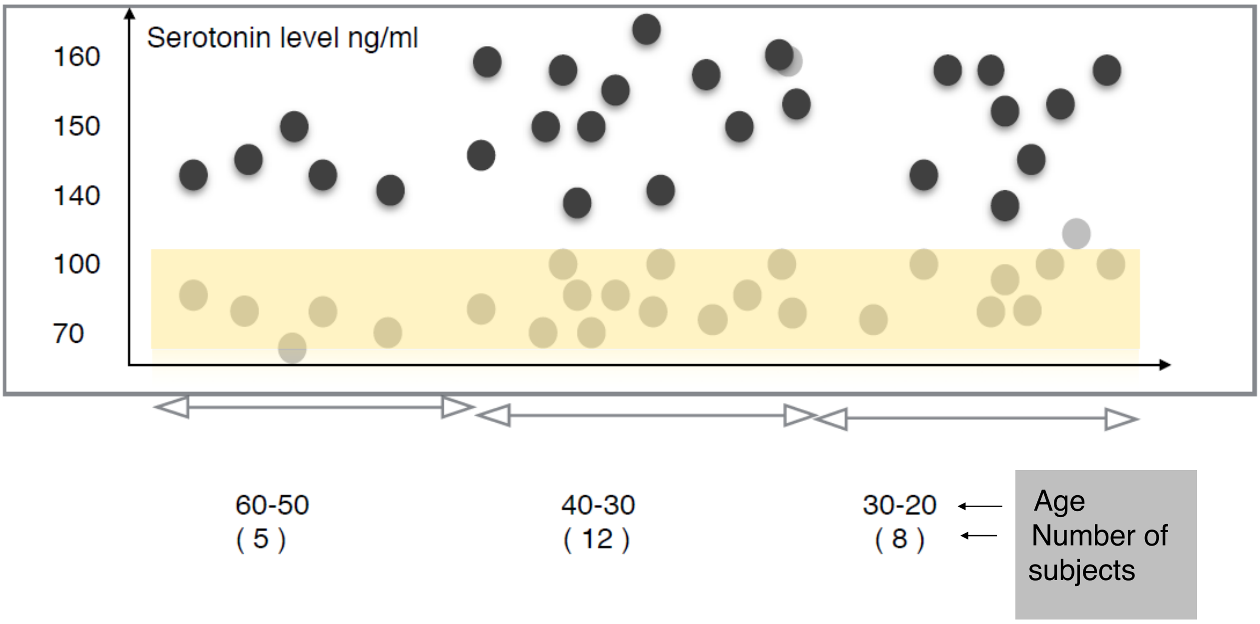
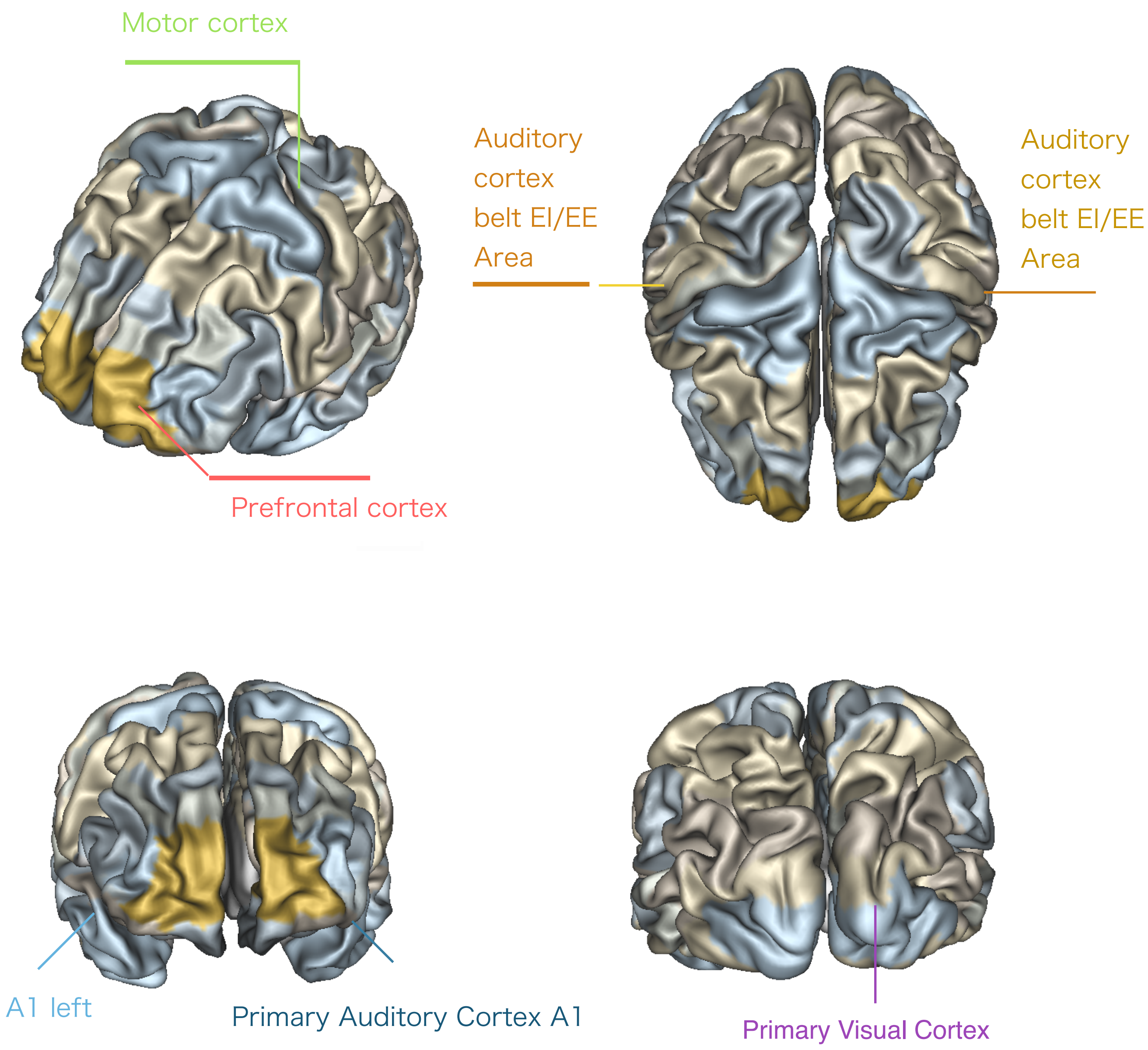


Chart No. 3. Age groups of patients and the level of serotonin in the tests of blood serum or plasma. The average level of HT-5 serotonin before and after 3-month dietary and physical activity programme to increase the level of BDNF - ICG-1 protein and HT-5 serotonin.

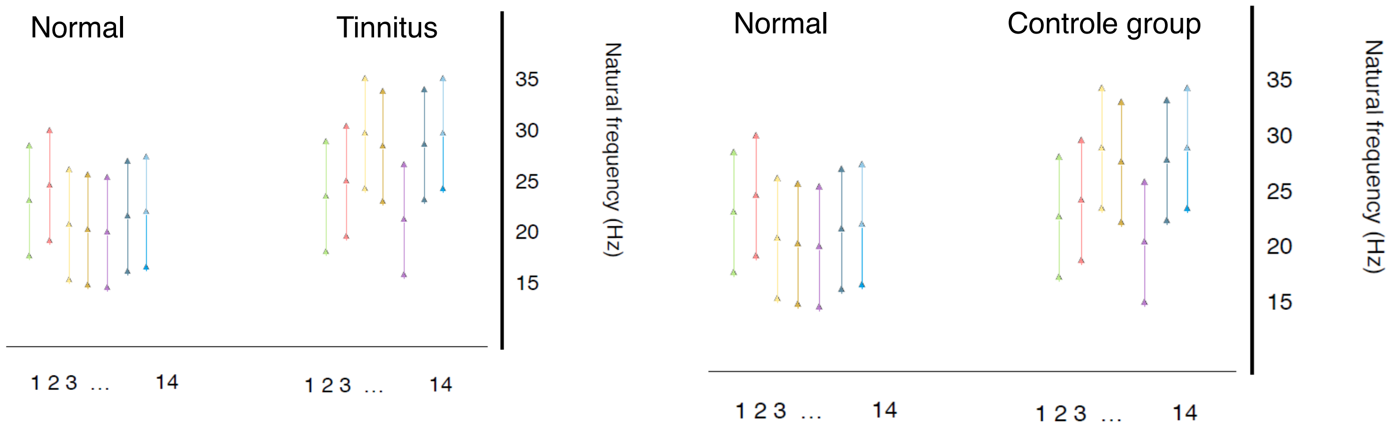
EEG measurment

The EEG test has wide applications in many fields of science. EEG test of Emotiv company was applied to measure the brain centers activity and their present oscillation (individual rhythmic of neurons) before beginning of the Arc synaptic adaptation therapy and also after 5-month period of the therapy. Source analyses of resting state encephalographic (EEG) signals of the classical EEG bands were correlated to subjective tinnitus perception scores measured on an Auditory Analogue Scale (AAS). In all 25 patients presenting strictly unilateral and bilateral tinnitus, AAS scores correlated positively with contralateral current source densities (CSD) in the primary and secondary auditory cortex in the high frequency range (beta 2 and gamma, max $r = 0.73$, $p<0.05$;) and with decreased ipsilateral parieto-occipital junction in the gamma band (max r = –0.72, p<0.05;). Delta CSD correlated negatively with VAS scores in contralateral temporal-occipital junction (max r = –0.74, p<0.05;). No significant correlations were found in the theta, alpha or beta 1 band. No significant correlations between gamma band activity and hearing loss, as measured by the loss in decibels (dB SPL) at the tinnitus frequency, were found using similar analysis as performed for tinnitus correlations.



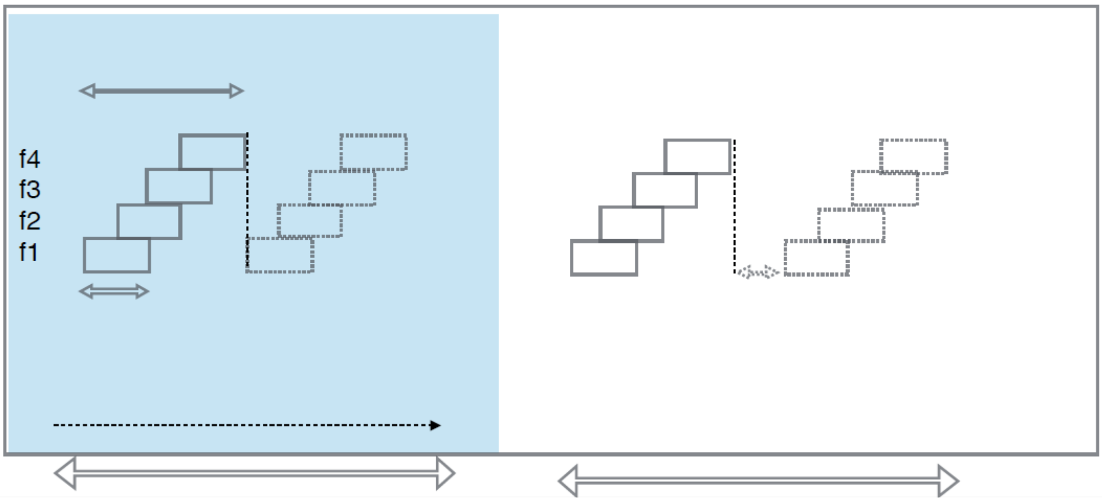
The above diagramme depicts the 3D model of the patient's brain and the centres which were tested with the application of the brain EEG of Emotive Company. The picture of the brain itself was created thanks to Syneika Imaging Sensory (Syneika Inc) neuronavigation. EEG signals were referenced to a forehead electrode, high-pass filtered (0.1Hz), and sampled at 1450 Hz electrode and the results of the test measurement.

EEG/ASSR in individual brain centers in patient with tinnitus average from the group of 25 patients (Chart No. 4.) and 6 controle group (Chart No. 6.)



Stimulation protocol together with the questionnaire

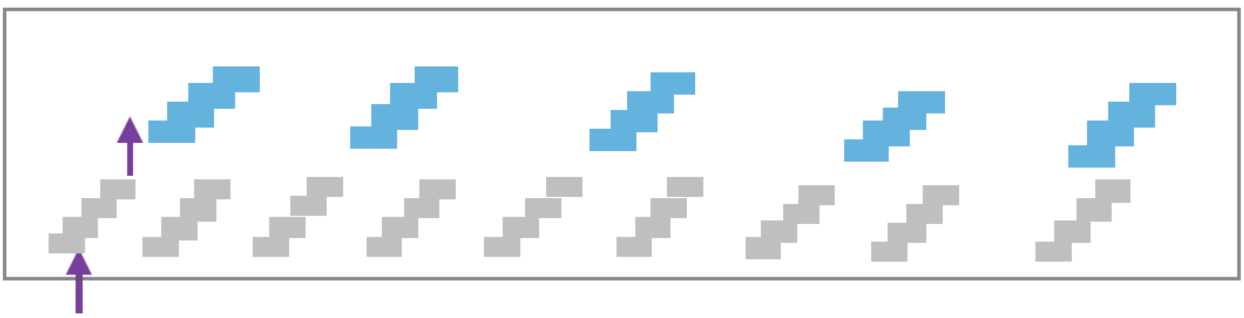
The device adjusted for this type of activities was used to map the effects and the influence of decreased stimulation in time, in frequency and intensity in accordance with gradual deprivation. Synaptic stabilizer manufactured by Bioaocustic sp.z.o.o Ltd Co. was used in the trial. The accoustic tones generator in the form of small portable device made up of DSP memory processor, loudness of the headphone input control and lithium battery. The device may be connected to a PC and be programmed, may be adjusted by computer software Synapsin1.0 (created by bioacoustic - videomed). The device may be programmed to generate the sequence of tones following each other (low – high). The device generates the sequence of tones connected with each other during one tone lasting (total time 100 ms) the next, higher tone enters within first 50 ms of the first tone lasting, the whole sequence is built in the similar manner. After producing the single sequence the loop turns on and it repeats the same sequence. The aim of the device is to introduce longer and longer interval between the first sequence and then the looped ones. The initial time between the first and next sequence is 0 ms, the device is our protocol was set up so that the time of the interval was longer by next 50 ms every week.



The first week of stimulation, the path is looped with the interval of 0 ms between the tones.

The second week of stimulation, the path is looped, with the 0 ms interval between the tones.

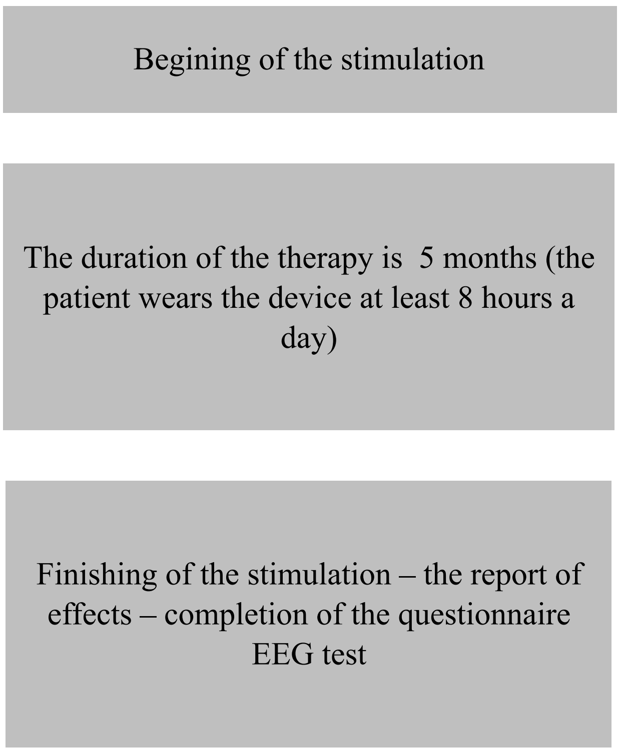
The above illustration presents the tones sequence (f1,f2,f3,f4) and the structure between individual tones and the time between them which changes every week of the therapy and is longer by subsequent 50 ms. In this manner the tone which gets less frequent in time with the lower and lower frequency of signal providing is obtained. Each individual tone lasts for 100 ms, combination of tones 250 ms. In the first week the tone sequence is presented one after another without any interval. After the first week the interval of 50 ms is added between the sequences which generates the effect of slowering the signal in time.



The level of basic stimulation of 30 dB, hearing threshold in the range of sudden loss of hearing for f1,f2,f3,f4.

The device may repeat the same tone algorithm in different levels of sound intensities (intensity octave - 6dB) which is why the algorithm may be stimulated not only in the plane of time and frequency but also in terms of dB intensity (SPL)The average of pathway intensity in dB (SPL), the intensities which are subject to reduction change every month. At the beginning they slower the loudest pathways in time (elongation of the interval), and then, after a month they are deleted from the stimulation programme to map the gradual loss of hearing also in terms of intensity. After deleting of subsequent louder pathway the one below which is set up by 6dB undergoes the process of signal slowering in time.

Each patient was given the device programmed in line with the range of their sudden loss of hearing, in which several tones covering the width of the band affected with the sudden death with different intensity with reference to loss of hearing were used, for example: loss of 30 dB 5 sessions of synapses intensity to the adaptation analyzing 6 dB of sound intensity were used. The calendar of set up changes at each patient, the changes of time elongation and slowering of the signal at the same time were introduced every 7 days.



Stimulation summary:

a) the stimulation protocol is built out of 4 tones, one tone lasted for 100 ms, each subsequent tone was given when the previous one still lasted; it was in the middle of the time - 50 ms

b) the device automatically, every week, lowered the stimulation level by 50 ms every week

c) the clock, in real time, every 7 days precisely, changed the level of stimulation, for example:

-11:00 28.05.2014 change of programme 11:00 03.06.2014 and so on, no matter how long did the patient wear the device everyday; the device changed the stimulations set up in the same interval of time (7 days).

d) It was recommended to wear the device of synaptic adaptation therapy everyday for 8 hours from the morning till the evening, along the natural cycle of blood temperature increase and also along with the increase of the activity of the central nervous system.

e) The patient received 24 forms in which they described every day the changes occurring. The form included the questions concerning the manner the patient hears tinnitus in the noise, whether it is less loud, how he hears the tinnitus in the silence, whether after certain period of stimulation the tinnitus was quieter or louder, whether the patient noticed any improvement or no changes, whether the tinnitus was less troublesome, less irritating, what was the level of their sleep.

f) The level of stimulation covered the scope of sudden loss of hearing within the width due to f1-f4 frequency in relation to the previous result of TT/TFR which indicated the period the patient did not hear the tinnitus. The tones were used and adjusted to the longest period of loss which show the maximum band of frequency which was affected by the loss of hearing and hyper-excitement of the tinnitus.

g) The level of intensity in dB for right and left ear in the scope of the sudden loss of hearing for the group with unilateral and bilateral loss of hearing were set up in the same manner, the patient, in spite of unilateral loss and the tinnitus heard the stimulation in the right and left ear so that not to allow for the negative migration of the neurons from one side to the other, and, by the same, not to worsen the representation of neurons on the side which was not stimulated and where there is no loss of hearing.

Tests results

After the completion of the therapy, after 5 months of stimulation the patients returned the devices. The data on the course of the therapy (how long did the patient wear the device) and in which time of the day were collected from the device. The list of the average from the data login Synapsin 1.0 indicates average use of the device in the range between 6 and 10 hours a day. While wearing the device the patients made breaks – nearly every hour a break of 30-40 minutes. The patients returned all forms they filled in during the therapy and were asked to fill in the TQ Global questionnaire used in the tests of therapy effects again. The patients underwent the EEG/ ASSR tests before and after the therapy which were to show the differences in the fields of hyper-excitement of nervous system.

TQ questionnaire

Among 25 examined persons 4 indicated the tinnitus as significantly reduced (90% assessment based on the tinnitus hearing and non-hearing during the whole day and night two weeks after taking off the stimulation), for 16 the tinnitus was reduced notably within 50-70% in TQ (Global) scale, 3 patients felt insignificant improvement in the tinnitus reduction (20-30 %), and 2 patients did not notice any change in the tinnitus reduction. The reduction effects are based on examination whether the patient hears the tinnitus in quiet room (audiology testing booth).

The below table depicts the effects of therapy in two different groups of patients; out of 10 persons with unilateral loss of hearing 8 persons felt the reduction in the range from 50-70 %, whereas two persons in this group did not feel any improvement. In the group of 15 people with bilateral loss of hearing - 4 persons felt the reduction at the level of 90 % in the relation to the scale of day and night, 8 persons felt the tinnitus reduction at the level of 50-70 %. The results are based on the final TQ Global questionnaire filled in by all patients.

Table of the effects of two	groups of patients of the tinnitus reduction	in silence.
Group	Tinnitus reduction 50-70%	Tinnitus reduction 0 %
10 persons with unilateral loss of hearing	8 persons	2 persons
	Tinnitus reduction 90 %	Tinnitus reduction 50-70 %
5 persons with bilateral loss of hearing	4 persons	8 persons

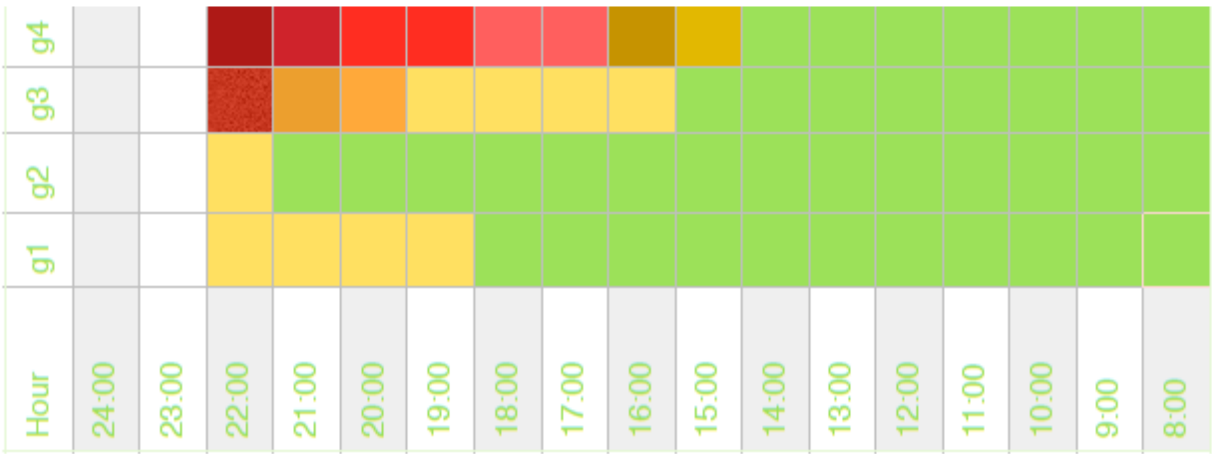
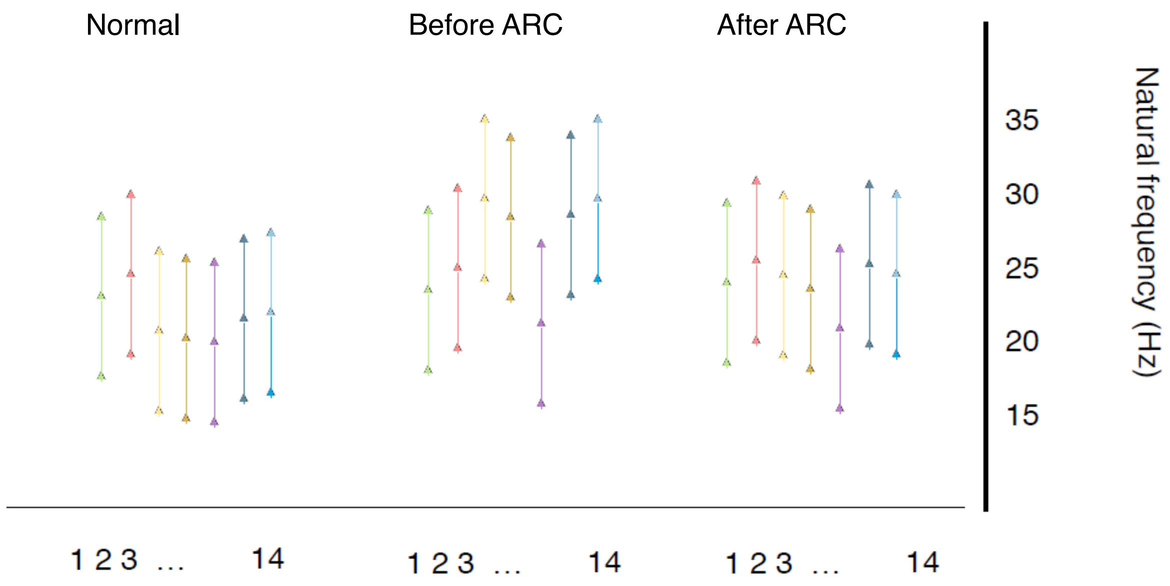


Table No.4

Table No.4 depicts the changes in hearing the tinnitus after the therapy during a day by all groups of patients g1,g2,g3,g4 (25). In all groups an explicit tinnitus reduction in the hours from morning till late afternoon is visible. In group g1 the influence of 5-month stimulation had it that after taking off the device the natural accoustic stimulation of the surrounding has a great impact on the tinnitus reduction, which is the evidence of a significant reconstruction of the cell-neuron innervation through the stimulation, 2 patients in this group reported the reduction at the level of 50 %, 3 persons reported the effect of reduction at the level of 70 % .In group g2 4 persons out of 8 reported the reduction result of 90 %, and 4 persons reported the result of tinnitus reduction of 70 %, the influence of TT/TFR stimulation in this group was the greatest, the persons in this group reported the quietest tinnitus and the best effect of stimulation were obtained in this group. This group of people indicated that in the evening they had to really focus their attention in silence to hear the tinnitus. Group g3 reported the greatest difference in the tinnitus discrimination at the background of other sounds of the surrounding after the stimulation. 3 persons out of 7 reported the result at the level of 30% of tinnitus reduction and significant reduction thereof during a day, 4 persons reported the reduction at the level of 60%. There were two persons in group g4 who did not report the effects of the tinnitus reduction in silence (0%) but noticed the tinnitus is inaudible for longer period of the day, 3 persons reported the reduction result at the level of 50%.

The EEG test results after the therapy

After 5-month Arc synaptic adaptation therapy EEG / ASSR tests were performed again; the results were as follows.



EEG fast frontal oscillation was reduced (0.32) before accoustic stimulation ARC

Neural oscillation after 5-month stimulation by Arc synaptic adaptation therapy for most of centers was decisively lower within the norm (healthy controls) without previously visible higher oscillations of primary and secondary auditory cortex visible in the preliminary EEG tests before the therapy, oscillation of the prefrontal lobe returned to norm which confirms the fact that this centre takes part in generating the tinnitus in most of patients (Sven Vanneste ,Kathleen Joos, Dirk De Ridder 2012).

Discussion

The tinnitus as the negative symptom of too quick deprivation of the nerve system of the stimulation is the most common problem in our society. In many cases the scientific environment associates the tinnitus with sudden loss of hearing suffered due to accoustic or mechanic injury. The aim of this trial was to show that the sudden loss of hearing need not relate to accoustic injury and may occur in any age group irrespectively the degree of exposure to noise. The research (Xiaouri Shi 2011) show that the external cells in the band from 4 to 20 kHz are less sensitive to oxidative stress in any form. Many research indicate that the reason of a sudden death of cells of the inner ear may be diabetes (K.E Elkind - Hirisch, W.R Stoner 2010), heavy drops of estrogen in women, and also the drops of serotonin which regulates the blood flow in women (Yanmin Luo, Premlata Kumar, and Carole R. Mendelson 2013), and the differences in the HDL and LDL cholesterol level (Ali A.Muttalib Mohammed 2014). Most of these factors of the risk of tinnitus and sudden loss of hearing occurring pertain to wider and wider group of persons mainly because of the pace of life, lack of suitable diet and physical activity and the increase of time spent sitting at work (drop of atrial natriuretic peptide – the risk of hypoxia and ischaemia of the inner ear, labyrinth). In spite of so wide epidemiology of tinnitus there are a few therapeutic solutions and additionally they are wrongly adjusted during the treatment. Not much research relating to the use of high frequency audiometry in the diagnostics of sudden loss of hearing and the correlation with the tinnitus can be found in the literature. The more so our trial is of a revolutionary character as we present therein the diagnostic application which makes the tinnitus diagnostic and the preciseness of its occurring in relation to the band of sudden loss of hearing possible with the use of at least high frequency and high correlation of other tests compliance (TT.TFR). The more so the tinnitus of each patient should be divided into different stages of its generation in relation to its duration in years. In the present trial our aim was to verify the influence of decreasing accoustic stimulation in time in patients with tinnitus which lasted shorter than two years. The effects of Arc synaptic adaptation therapy are promising pursuant to TQ questionnaire and EEG tests, however the therapy itself is built from quite simply parameters and it would require further examination of variables to improve the stimulation protocol by introducing additional parameters of variables, such as the stimulation of EE and EI neurons (Excability Excabliti, Excability Inhibitor). The authors of the therapy reserve that the stimulation itself is created with the aim to adapt many bands of synapses and neurons which analyze the intensity and different frequency in the peripheral auditory pathway (cochlear nucleus) and in fact it is. However, an additional algorithm should be included in the protocol whose task would be to depolarize also the neurones in the auditory cortex EE, EI. In spite of many limitations related to the tests and the technology itself it was possible to achieve initial effects of therapeutic strategy which may have impressive effects if it would be adjusted to proper model and the place of generating the tinnitus in patient. Bioacoustic, as the author of the technology reserves additionally that the therapy must not be applied in patients with lower plasticity, it is the proper strategy as the research (Owen M.Wolkowitz ,Jessica Wolf 2011) actually indicate that BDNF protein and HT-5 serotonin may be the indicator whether the therapy would show the effect and what changes may be foreseen. This, however, generates the limitation in the form of the number of people who may be qualified to such type of therapy. Among 100 persons 25 could be qualified due to too low level of HT-5 serotonin. The treatment process may be long-lasting due to the attempt to lead the neurotransmission to a proper level. Additionally, the therapy of such type may be applied only in the model of spontaneous activity (Dave R. M. Langers, Emile de Kleine and Pim van Dijk 2012) which must be preceded by suitable tests to confirm the model where the neuron and the cell are still connected. From the logical point of view the effectiveness of this therapy may be small with the strong deprivation of innervation and in patient with the tinnitus of the second model (enhanced synchronization), in that case, in turn, other effective strategy of reduction, for instance rTMS may be applied. Summing up, the present trial was aimed at initial testing the effectiveness of Arc synaptic adaptation therapy and checking whether the diagnostic model chosen for this technology has logical basis and may be used in everyday diagnostics and analysis in patients with tinnitus. The therapy itself requires the preparation of the patient with regard to the proper level of neurotransmitters, and by the same, high level of plasticity and properly conducted diagnostics which shall determine the place of sudden loss of hearing connected with the death of external cells in the inner ear, and by the same also the tinnitus generation in the peripheral auditory pathway. The therapy of synaptic adaptation may be an effective solution of treatment of tinnitus in the initial phase of tinnitus generating in the model of spontaneous activity up to 2 years, simultaneously in patients with unilateral and bilateral loss of hearing. The only therapeutic limitation of the presented above solution may be the width of band of sudden loss of hearing (algorithm which is built out of many tones losses natural speed of repeatedness, thus the signal is slower than in case of shorter sequence of tones – more narrow loss of hearing) and the stimulation scope (the loss of hearing must not be within speech recognition from 125 Hz to 10 kHz but above). More precise research relating to different set up of time of signal slowering and the intensity to different width and the depth of the loss of hearing is needed to determine the most efficient variant of the stimulation. The most important element of the effectiveness of the therapy that was examined is decisively statistically less and less frequent signal and decreasing intensity of stimulation. In the control group composed of 6 persons after the application of 5-month stimulation in the scope of sudden loss of hearing by the signal which does not change its parameters during the therapy no changes were noted in the reduction of tinnitus when questionnaire TQ is concerned and the activity of the oscillation of the centers in brain in EEG tests which confirms the Bioacoustic theories on the influence and the effectiveness of repeated trial of deprivation mapping thanks to stimulation decreasing in time. For a long time in the scientific environment it was discussed that the amount of spontaneous activity released by synapses and the neuron activity and morphology depend upon the statistics of the signal occuring in time in a determined band of stimulation (Muhammed S. A. Zillany and Laurel H. Carney 2011), which additionally confirms the proper direction of the work on Arc synaptic adaptation therapy as the effective manner to reduce the tinnitus in future.

Clinical trial accepted by Ethics Committee at the Nencki institute of Experimenta Biology in Warsaw Poland KI 26312014 (KT 189022014)

Reference

1.The Reduced Cochlear Output and the Failure to Adapt the Central Auditory Response Causes Tinnitus in Noise Exposed Rats 2012 Lukas Ruttiger. Wibke Singer. Rama Panford-Walsh., Masahiro Matsumoto, Sze Chim Lee, Annalisa Zuccotti, Ulrike Zimmermann, Mirko Jaumann, Karin Rohbock, Hao Xiong, Marlies Knipper

2.Arc/Arg3.1 Mediates Homeostatic Synaptic Scaling of AMPA Receptors 2006 Jason D. Shepherd, Gavin Rumbaugh, Jing Wu, Shoaib Chowdhury, Niels Plath, Dietmar Kuhl, Richard L. Huganir, and Paul F. Worley.

3.Progressive Degradation and Subsequent Refinement of Acoustic Representations in the Adult Auditory Cortex 2003 Shaowen Bao, Edward F. Chang, Jonathan D. Davis, Kevin T. Gobeske, and Michael M. Merzenich Keck Center for Integrative Neuroscience, University of California, San Francisco, California 94143-0732

4.Estrogen influences auditory brainstem responses during the normal menstrual cycle 1992 K.E Elkind-Hirsch, W.R Stoner, B.A Stach.

5.Potential Hormonal Mechanisms of Attention-Deficit/Hyperactivity Disorder and Major Depressive Disorder: A New Perspective 2009 Michelle M. Martel, M.A.1,2, Kelly Klump, Ph.D.2, Joel T. Nigg, Ph.D.2, S. Marc Breedlove, Ph.D.2, and Cheryl L. Sisk, Ph.D.

6.Estrogen-Related Receptor Regulates Oxygen-Dependent Expression of Voltage-gated Potassium Channels and Tissue Kallikrein during Human Trophoblast Differentiation 2013 Yanmin Luo, Premlata Kumar, and Carole R. Mendelson

7. A clinical study of serum lipid disturbance in Chinese patients with sudden deafness 2013 Tingwen Weng, Erin E Devine, Hongming , Zhisong Yan and Pin Dong

8.Dyslipidemia and Auditory Function 2006 M. Bradley Evan, Ross Tonini, Cynthia Do Shope, John S. Oghalai, James F. Jerger, William Insull Jr.‡, and William E. Brownell.

9.Lipid Profile among Patients with Sudden Sensorineural Hearing Loss 2014 Ali A. Muttalib Mohammed

10.Brain-Derived Neurotrophic Factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis 2013 Sharain Suliman, Sian M. J. Hemmings and Soraya Seedat

11.Serum BDNF Levels Before Treatment Predict SSRI Response in Depression 2011 Owen M. Wolkowitz, , Jessica Wolf, , Wendy Shelly, , Rebecca Rosser, , Heather Burke, P George K. Lerner, MD1, Victor I. Reus, , J. Craig Nelson, , Elissa S. Epel, PhD, and Synthia H. Mellon, PhD

12.Regulation of extracellular serotonin levels and brain-derived neurotrophic factor in rats with high and low exploratory activity 2008 Tanel Mallo, Kadri Koiv

13.Serotonin transporter and BDNF polymorphisms interact to predict trait worry 2014 Bredemeier K, Beevers CG

14.Brain-Derived Neurotrophic Factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis 2013 Sharain Suliman Sian M. J. Hemmings and Soraya Seeda

15.Tinnitus does not require macroscopic tonotopic map reorganization 2012 Dave R. M. Langers Emile de Kleine, and Pim van Dijk

16.Dynamic Range Adaptation to Sound Level Statistics in the Auditory Nerve 2009 Bo Wen, Grace I. Wang Isabel Dean and Bertrand Delgutte

17.Power-law dynamics in an auditory-nerve model can account for neural adaptation to sound-level statisticsc 2010 Muhammad S. A. Zilany and Laurel H. Carney

18.Rapid Neural Adaptation to Sound Level Statistics 2006 Isabel Dean, Ben L. Robinson, Nicol S. Harper, and David McAlpine

19. Frontal Cortex TMS for Tinnitus 2014 Dirk De Riddera,, Jae-Jin Songa,b, Sven Vanneste