Physiological regulation of chronic tinnitus: A new methodological approach

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Abstract-English

Decrease of auditory alpha-rhythm might lead to tinnitus. This theory calls for a rehabilitation strategy using training to increase auditory alpha in chronic tinnitus patients. A novel auditory alpha monitoring method was devised to circumvent the inverse problem of standard EEG source localization methods and low signal to noise ratio. To prevent projection of alpha from somatosensory and occipital cortex, alpha-blockade of these two regions using tactile and visual stimulations is integrated in an EEG neurofeedback set-up. The monitoring online source method's results of the first study showed rigorous tracking of auditory alpha activity purely from the sensor-level. Furthermore, the pilot experiment realized that up-regulation of auditory alpha activity is very difficult for tinnitus patients. Therefore, it was decided to use only contingent feedback and to document the neurofeedback's effects of auditory alpha on the behavioral and neurophysiological level and not using a control condition or control group. Instead a learner and not learner group was differentiated. The neurofeedback training exerts a positive effect on the tinnitus symptoms reducing distress level in all recruited patients. However, this effect is not a consequence of the physiological changes induced by localized alpha neurofeedback in auditory cortex. It seems to be an unspecific psychological consequence probably of positive expectancy and the reduction of cognitive dissonance related to the attribution of concentration and effort. Only a few patients, independent of the severity of the symptoms and accompanying psychological disorders learn to control and increase auditory alpha activity at a significant level: Different reasons could be responsible for this lack of covariation between neurophysiology and behavior: 1) training time too short for automatization of learned alpha increase in the real environment (generalization) and/or permanent neuronal reorganization of the auditory structures after the many years of tinnitus unresponsive to a non-invasive training procedure. 2) Auditory alpha is not causally responsible for tinnitus, thus learned changes of alpha have no effect on tinnitus. However, the developed on-line neurofeedback of localized alpha allows systematic replication and variation of the determining variables of neurofeedback training in tinnitus in future larger clinical trials. On the productive side, our results strengthen a physiological origin of tinnitus and do not support a causal relationship between psychological factors ("stress") and tinnitus. Rather they strongly point toward the psychological suffering (stress, anxiety and depression) as a consequence of the sound sensation and its physiological causes of unknown origin at present. The future will confirm or invalidate our explanation, but we would be surprised if psychophysiological theoretical explanations such as Jastreboff's popular Tinnitus model turns out to be correct.

Abstract-Deutsch

Eine Reduktion des auditiven Alpha-Rhythmus kann kausal zu Tinnitus führen. Diese Theorie wird in einer Rehabilitationsstrategie mit Training des auditiven Alpha-Rhythmus bei chronischen Tinnitus-Patienten angewandt. Eine neuartige Methode zur Rückmeldung und online Lokalisation des auditiven Alpha-Rhythmus wird entwickelt, um das inverse Problem von Standard-EEG-Quellenlokalisierungsmethoden und niedrigem Signal-Rausch-Verhältnis zu umgehen. Um den Einfluss von Alpha aus dem somatosensorischen und okzipitalen Kortex zu verhindern, wird die Alpha-Blockade dieser beiden Regionen mittels taktiler und visueller Stimulation in ein EEG-Neurofeedback-Setup integriert. Die Ergebnisse der ersten Studie mit der Monitoring-Source-Methode zeigten eine rigorose Darstellung der auditiven Alpha-Aktivität aus der Sensorebene allein. Darüber hinaus zeigte sich, dass eine Hochregulation der auditiven Alpha-Aktivität für Tinnitus-Patienten sehr schwierig ist. Daher beschlossen wir, nur kontingentes Feedback zu verwenden und die Auswirkungen des Neurofeedbacks auf das Verhalten und die neurophysiologische Ebene zu dokumentieren und keine Kontrollbedingung oder -gruppe zu verwenden. Nur zwischen Lernern und Nicht-Lernern wurde differenziert. Das Neurofeedback-Training übt einen positiven Effekt auf die Tinnitus-Symptome aus und reduziert das Leiden bei allen rekrutierten Patienten. Dieser Effekt ist jedoch nicht eine Folge der physiologischen Veränderungen, die durch das lokalisierte Alpha-Neurofeedback im auditorischen Kortex induziert werden. Es handelt sich um eine unspezifische psychologische Folge wahrscheinlich der positiven Erwartungshaltung und der Verminderung kognitiver Dissonanzen im Zusammenhang mit der Zuschreibung von Konzentration und Anstrengung. Nur wenige Patienten lernen, unabhängig von der Schwere der Symptome und der begleitenden psychischen Störungen, die auditorische Alpha-Aktivität zu kontrollieren und signifikant zu steigern: Verschiedene Gründe könnten für diese fehlende Kovariation zwischen Neurophysiologie und Verhalten verantwortlich sein: 1) eine zu kurze Trainingszeit für die Automatisierung der Alpha-Zunahme in der realen Umgebung (Generalisierung) der Patienten und/oder eine permanente neuronale Reorganisation der auditorischen Strukturen nach den vielen Jahren des Tinnitus, der auf ein nicht-invasives

Trainingsverfahren nicht anspricht. 2) Der auditorische Alpha ist nicht kausal für den Tinnitus verantwortlich, so dass erlernte Veränderungen der Alpha-Aktivität keinen Einfluss auf den Tinnitus haben. Das entwickelte Online-Neurofeedback von lokalisiertem Alpha erlaubt jedoch eine systematische Replikation und Variation der bestimmenden Variablen des Neurofeedbacktrainings bei Tinnitus in zukünftigen größeren klinischen Studien. Auf der produktiven Seite verstärken unsere Ergebnisse einen physiologischen Ursprung des Tinnitus und sprechen nicht für keine kausale Beziehung zwischen psychologischen Faktoren ("Stress") und Tinnitus. Vielmehr weisen sie stark auf das psychische Leiden (Stress, Angst und Depression) als Folge des Tinnitus Geräusches und seiner physiologischen Ursachen derzeit unbekannten Ursprungs hin. Die Zukunft wird unsere Erklärung bestätigen oder entkräften, aber wir wären überrascht, wenn sich psychophysiologische theoretische Erklärungen wie das populäre Tinnitus-Modell von Jastreboff als richtig erweisen sollten.

To my parents, without whose faith,
encouragement, and understanding
throughout my life this thesis
would never have been written.

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Chapter 1

I. Theoretical Part

1 From Sound to Neural Signals

1.1 Sound

Sound is a mechanical vibration which alters the pressure (measured in Pascal (Pa)) in a sound-transmitting medium, like air. Sound can be described mathematically in two domains, time and frequency. In the temporal domain, a sound is represented by a function of the pressure over time (t) and can be described by a single sinusoidal wave if it is a pure tone, or as a summation of sinusoidal functions if it is a complex sound. Amplitude in time domain refers to the sound pressure. In the frequency description, sound is defined in terms of the frequency components that make up the sound. Below some characteristics and measures are defined to describe sound which in the following chapters would be used.

- Sound pressure (p): Sound pressure is the sound force (N) acting on the surface area (m^2) perpendicular to the direction of the sound: p=F/Ar(N/ m^2)
- Sound intensity (I): sound intensity defines as the power per unit carried by a wave. Mathematically $I = p^2/p_o$, where p is the sound pressure in sound-transmitting medium (e.g., air), and p_o is the density (p_o) of the sound-transmitting medium.
- Decibel (dB) scale: As human ear is sensitive to wide range of sound frequency(20-20000Hz) nonlinear representation such logarithmic scale is used to express this wide range of human ear sound capability. Decibel is defined mathematically based on the logarithmic scale:

dB = $10*\log_{10}(p/p_{ref})$, where p is sound pressure, ref is defined as a reference value, and $\log_{10}$ is the base-10 logarithm. Here p_{ref} is 20 micro pascals and the measure of decibel is expressed as dB (sound level).

1.2 Auditory system

Process of acoustic stimuli occurs in auditory system which it is one of the sensory systems. Generally, this system has two parts: the periphery includes outer ear, middle ear and inner ear, the central part begins from the cochlear nucleus and ends at the primary auditory cortex.

1.2.1 Peripheral Auditory System

Processing of acoustic stimuli begins with traveling sound waves through the auditory canal which is a part of the outer ear. Incoming sound waves vibrate the tympanic membrane or eardrum which is located at the end of the auditory canal. Vibration of eardrum then moves the ossicles, three tiny bones in the middle ear called the malleus, incus, and stapes. Stapes conducts the energy sound to a membrane-covered known as oval window, which located in the wall of the cochlea (Figure 1-1 A). The cochlea is structure in the inner ear that resembles a snail shell which is for auditory transduction.

The cochlea is comprised of three parallel canals: the scala vestibuli, the scala tympani and the scala median (cochlear duct). The first two are filled with perilymph fluid and the last one with endolymph fluid. The first two canals are responsible for the transmission of pressure. The third called organ of Corti, has two different types of mechanosensory cells, known as hair cells, outer and inner hair cells which positioned on a thickened ridge of the basilar membrane. Basilar membrane located inside cochlear. It has more stiffness close to the oval window and floppiest neat the apex. This stiffness variation of basilar membrane works as spectral analyzer. High frequency sound resonates the part of the basilar membrane closing to the oval window and excitation of nerve cells sites in that part. In the same manner low frequency sounds leads to the excitation of nerve cells at the close to the apex (Figure 1-1 B).

Three outer hair cells and one inner hair cells are positioned at each segment of the basilar membrane. Mechanical amplification of low-level sound which enters the cochlea is carried out by the outer hair cells whereas the inner hair cells transduce the sound vibrations via the basilar membrane vibration into electrical activity that is then relayed via the auditory nerve to the auditory brainstem and to the auditory cortex (Figure 1-2).

Generally, vibration of the Oval window creates waves that goes through the fluid of the cochlea resulting in moving the basilar membrane. The basilar membrane functions as a spectral analyzer which converts the frequency of sounds entering to the ear into neural activity that can then be sent to the brain. This translation occurs in the organ of Corti. On top of each inner hair cell is a collection of small "hairs" called stereocilia. When the basilar membrane vibrates, this causes movement of the hair cells and their stereocilia; movement of the stereocilia opens ion channels and causes the release of neurotransmitters which then create the electrical impulse to propagate the auditory signal to the auditory vestibular nerve, which it carries the auditory information to the central auditory system in the brain to be analyzed and perceived[1].

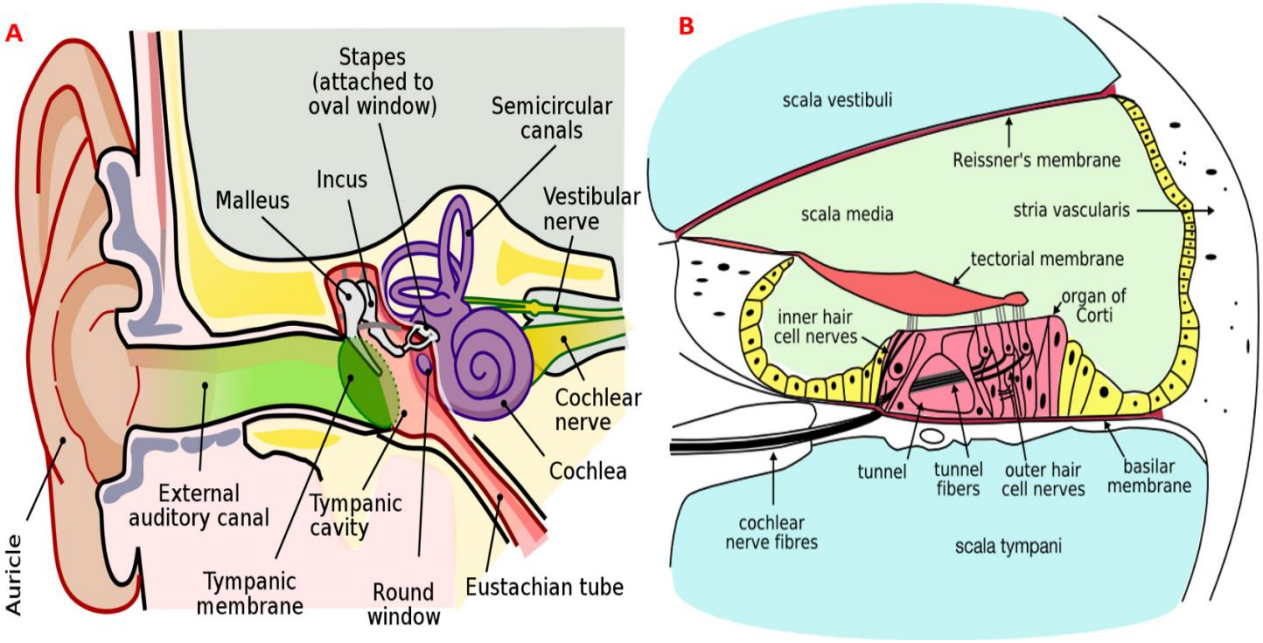

Figure 1-1:Peripheral auditory system. A) Outer ear (brown), middle ear(red) and inner ear (purple) and B) Cross section of cochlear (see text for further description). Figure A from : https://commons.wikimedia.org/wiki/File:Anatomy_of_the_Human_Ear.svg is licensed under cc BY 2.5 Figure B from : https://commons.wikimedia.org/wiki/File:Cochlea-crosssection-de.png is licensed under CC BY-SA 3.0.

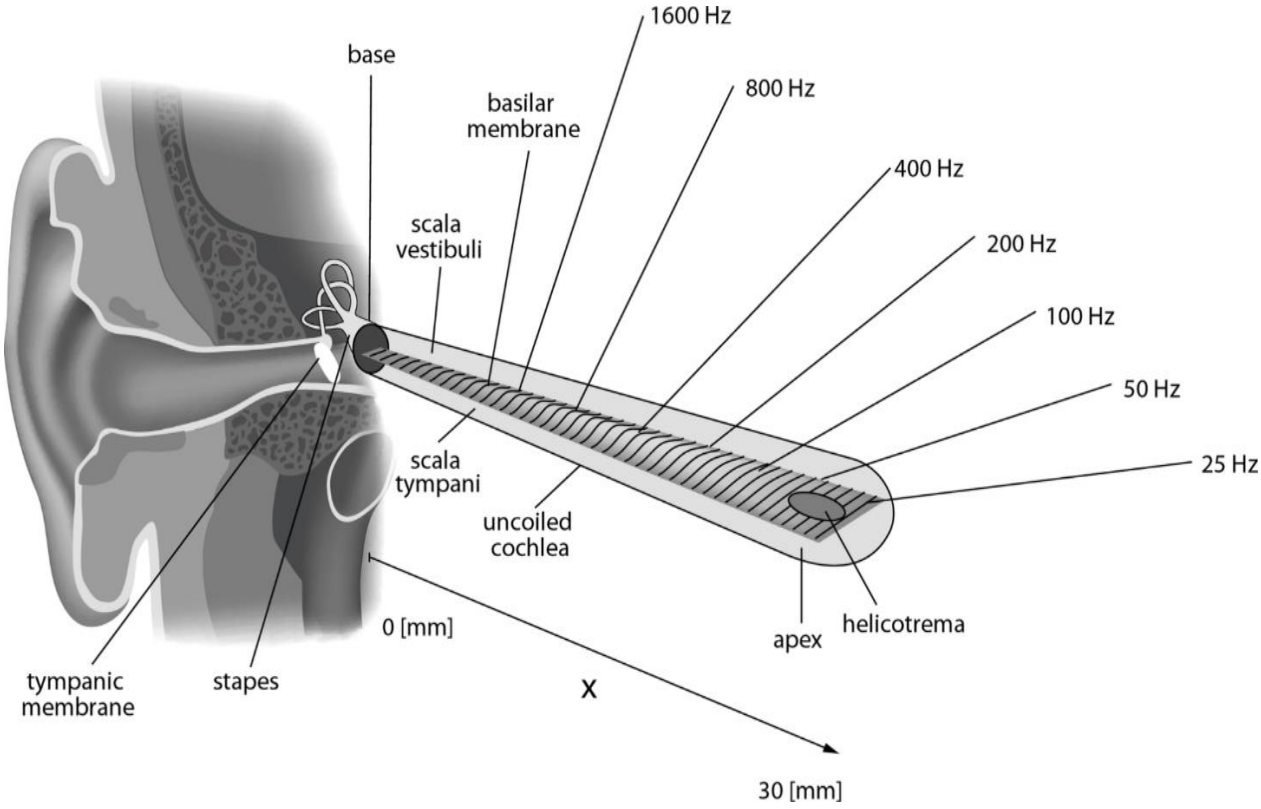

Figure 1-2: Uncoiled cochlea with basilar membrane. The analysis of sound frequencies occurs firstly at the basilar membrane. Each area of the basilar membrane vibrates selectively to a particular sound frequency. High-frequency sound waves cause maximum vibration. Figure from https://commons.wikimedia.org/wiki/File:Uncoiled_cochlea_with_basilar_membrane.png is licensed under cc BY 2.5.

1.2.2 Central Auditory System

The auditory information from cochlea to the primary auditory cortex are conveyed through two pathways: vestibulocochlear nerve (CN VIII (*eighth cranial nerve*)) known as primary auditory pathway which only carries pure auditory information, and the non-primary pathways (also called the reticular sensory pathway) process all sorts of sensory messages. The main function of this pathway is to select the type of sensory input to be treated first.

1.2.2.1 Primary auditory pathways

Each auditory nerve in left or right cochlear has approximately 25000-30000 nerve fibers. Auditory nerve's fibers are axons of a spiral ganglion cells conveying a particular frequency information of sound and a particular range of loudness. Two characteristics

of each nerve fiber represent this information: 1) rate of action potentials 2) timing (rhythmicity) of individual action potentials. The primary auditory pathway has 4 relays nuclei, starting from cochlea and ends in the primary auditory cortex. Each relay nucleus involves specifically in decoding and integration of auditory input. The first brain structure of this pathway is the **cochlear nuclei** located in the brain stem, which receives ipsilaterally type I spiral ganglion axons (auditory nerve) which exclusively innervate the inner hair cells (Figure 1-3).

Brainstem cochlear nuclei consist of two cranial nerve, the ventral cochlear nucleus (VCN) and the dorsal cochlear nucleus (DCN). Processing of the time information of neural firing and the activation pattern of the neural population of auditory nerve fibers occurs in VCN. The DCN has several roles, such as encoding spectral monaural cues related to the pinna movements (the visible part of the ear located outside the head), selecting what to hear based on the salience, behavioral state (and suppressing hearing responses to self-produced sounds), providing coordination with other sensory related systems (vestibular, somatosensory,...) to localize sounds. The other important characteristic of VCN and DCN is that they are tonotopically organized, with DCN regions receive auditory nerve fibers that arise from apical parts of the cochlea, sensitive to high sound frequencies, and ventral cochlear nucleus regions receive auditory nerve fibers arising from the basal (low frequency) part of the cochlea which is tuned to low sound frequencies.

The second major relay nucleus located in the medulla is the **Superior Olive** (Figure 1-3 A). This structure is constituted of a arrange of nuclei named as the **superior olivary complex**. Superior olives of each side receive both the ipsilateral and contralateral cochlear nucleus projections. Each primary auditory cortex is corresponded mainly to contralateral auditory sound information because the contralateral projection is largest in each superior olive. The medial and lateral superior olive nuclei process the auditory input in two distinct ways which this distinctive information projects to the next relay to localize the sound. The medial part of the superior olive acts on differences in the time in which a given stimulus arrives at each of the ears. But the lateral section responds to amplitude differences of acoustic waves reaching each ear. Tonotopic organizations of

each two mentioned parts of the superior olivary complex, the medial and lateral subdivisions, have been reported [2].

The third rely is the **inferior colliculus** (mesencephalon), the primary auditory midbrain, located in the brainstem (Figure 1-3). All auditory pathways traveling through the brainstem converge in the inferior colliculus . From this structure auditory information also projects to the other areas like the superior colliculus or thalamus. From number of auditory and non-auditory regions such as cochlea itself and the superior olivary nuclei information protrude to the inferior colliculus. The inferior colliculus roles in integration of auditory information and fine-tuning the same information is very important.

The inferior colliculus is organized anatomically by three divisions, the central nucleus, the dorsal cortex, and the external cortex. The nuclei in the central part are organized tonotopically[3]. The inferior colliculus neurons, like the superior olivary nuclei, are functionally organized to be responsive to auditory signals (such as amplitude, the difference in arrival time of a sound from the both ears). These characteristic gives the capability to the inferior colliculus to localize the sound, or to determine where in space, sound is coming from. Due to the fact that there is a pathway between the inferior and superior parts of the colliculus, localization information is relayed to the superior colliculus, which responds to visual and auditory cues in the environment by orienting the eyes and head. Animal studies have shown changes in visual gaze directions modifies concurrent auditory processing. It has been demonstrated that eye position in macaques changes the firing rates of auditory neurons at the inferior colliculus[4]. These connectivity between the inferior colliculus and cortical areas could moderate complex gaze control related tasks that incorporate aspects of memory, recognition. However, there is also an additional important characteristic of the external nucleus of the inferior colliculus. The inferior colliculus external nucleus has ascending projections from both auditory and somatosensory nuclei. Auditory and somatosensory system representation in the external nucleus of the inferior colliculus in the cat has been shown. This representation of these two different systems in external nucleus could explain the involvement of somatosensory system in tinnitus[5].

The last obligatory relay for all ascending auditory information before the auditory cortex is the **medial geniculate body** which is located in the thalamus (Figure 1-3 A). This relay receives auditory input from the inferior colliculus and some from lower auditory brainstem structures directly. The medial geniculate complex receives convergent inputs of spectrally and temporally distinct pathways. This complex by virtue of its convergent inputs provides recognition of specific spectral and temporal combinations of sounds. In several species, including humans, different spectral and time cues are notably important features of speech communication sounds. It is not known if human medial geniculus cells are selective for sound combinations, but speech processing certainly requires spectral as well as temporal combination sensitivity. This is where an important type of integration occurs: the preparation of a motor response (e.g., a vocal response).

The final station of the afferent primary auditory pathway is the auditory cortex (Figure 1-3 B). Auditory cortex is functionally divided to three main subregions. 1) primary auditory cortex(AI) 2) Secondary auditory cortex (AII) and 3) The belt region. The A1 is located on the superior temporal gyrus and receives input from the ventral part of the medial geniculate body. The primary auditory cortex is precisely tonotopically organized representing topographical map of the cochlea. The primary visual cortex (V1) and the primary somatic sensory cortex (S1) also possess topographical maps of their associated sensory inputs. But there is distinctive feature of auditory system comparted to vision and somatosensory. The decoding of the auditory stimulus already occurs in the cochlea, which is tonotopically located along the basilar membrane. The secondary auditory cortex has an essential role in sound localization and the analysis of complex sounds such as speech. It receives projections from the superior olive complex and the inferior colliculus of the midbrain. Sound localization occurs here through two specific mechanisms. The information related to time differences of sound arriving to the two ears which projects to the AII through medial superior olivary complex. The information of the differences in intensity of sounds arriving the eras projects to the AII via the lateral superior olivary complex. AII via this two auditory information localizes the sound in space. It should be mentioned that the AII doesn't have clear tonotopic organization because it receives input from the belt area of the medial geniculate body. It also serves a function in auditory

memory. The belt region which is surrounding AI and AII has a role in integration of auditory information with other sensory systems.

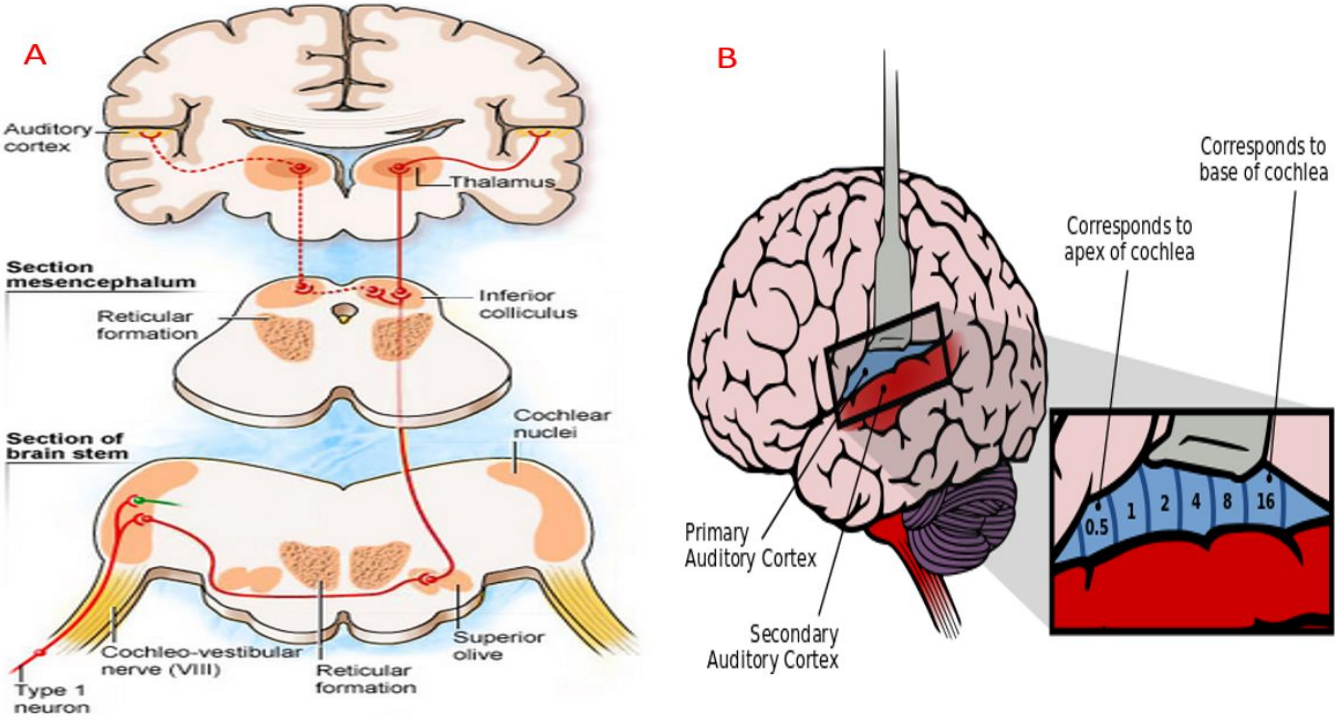

Figure 1-3: A) Primary auditory pathway and B) Auditory cortex. Auditory information travels from the receptors in the organ of Corti of the inner ear (cochlear hair cells) to the central nervous system through vestibulocochlear nerve (CN VIII) which consecutively passes four relays , cochlear nuclei, superior olive, inferior colliculus and medial geniculate body and finally reaches to the last station of auditory system, auditory cortex. The auditory cortex is located in the superior temporal gyrus has two main parts**,** the primary auditory cortex (A1) which is organized tonotopically, and secondary auditory cortex (A2) which is not tonotopically organized, but its main role in auditory system is sound localization. Figure A from http://www.cochlea.eu , Figure B from https://en.wikipedia.org/wiki/File:Auditory_Cortex_Frequency_Mapping.svg is licensed under cc BY 2.5.

1.2.2.2 Non-primary pathways

Short fibers from cochlear nuclei connect with reticular formation where the auditory information integrate with the other sensory and efferent information (Figure 1-4 A). The reticular pathways has connection with the wake and the motivational areas to prioritize or suppress the information. The next relay in this pathway is located in the non-specific reticular thalamic nuclei. A distinct feature of non-lemniscal neurons is that they send a strong efferent projection to a number of limbic structures such as amygdala, insular-

temporal lobe (associational auditory cortices) and striatum[6]. The core function of non-primary auditory pathway is to select the type of sensory information to be treated first. This function allows the person to focus on the most vital task while performing multitasks, such as reading a book and listening to a music in which the main focus is on reading the book.

Conscious perception of auditory input is a result of integration of both pathways (Figure 1-4 B). One good instance manifests itself during sleep. Due to inactive link between reticular pathways and the wake and motivation centers while sleep conscious perception of auditory input does not occur. In patient with trauma which affects the cortex leading to the suppression of the conscious perception the startle reflexes are still present. The reason is continuing integrity of the non-primary pathways resulting in vegetative reflex reactions to a sound.

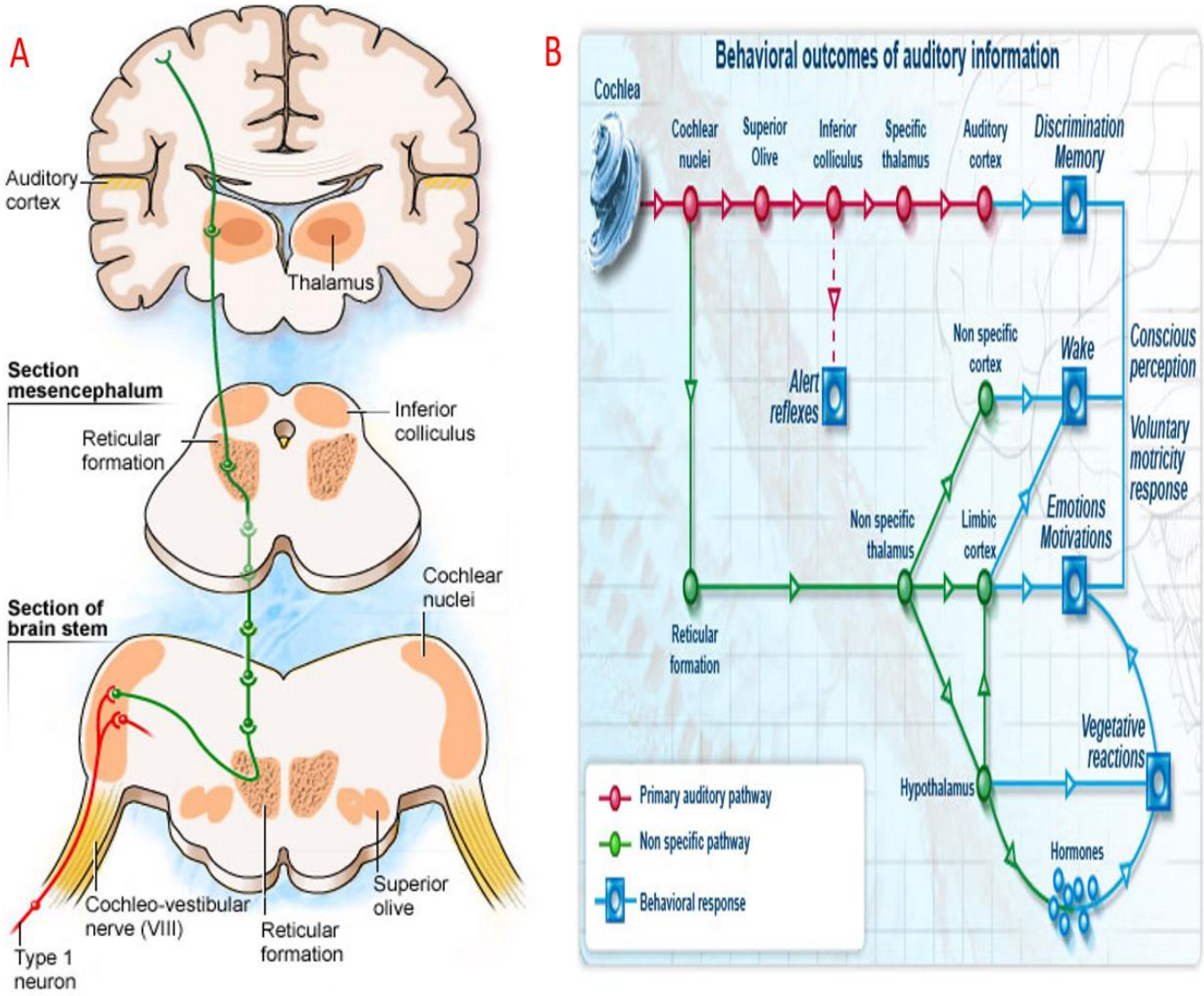

Figure 1-4: A) Non-primary auditory pathway and B) integration of both pathways (primary and non-primary auditory) and behavioral outcomes of auditory information. Both figures A and B from http://www.cochlea.eu.

Chapter 2

2 Tinnitus

2.1 Subjective vs Objective Tinnitus

The word tinnitus derives from the Latin **tinnire** meaning 'to ring', and in English is defined as 'a ringing in the ears'. Two main types of tinnitus exist - objective tinnitus and subjective tinnitus. Objective tinnitus characterized as a perceived sensation of noise that arises in the absence of external acoustic stimulation but can be also heard by the audiologist placing a stethoscope over the patient's external auditory canal. The source of objective tinnitus can be in vascular, muscular, skeletal, or respiratory structures[7]. The most prevalent objective tinnitus is pulsatile tinnitus[8] that can be synchronous with the cardiac beat[9], in which case a vascular source is likely, or non-synchronous, in which case myoclonus of middle-ear or palatal muscles is likely[10-12]. In the case of a muscular origin, such as contractions of palatal or middle ear muscles, the sound heard by patient is often described as "clicking" noise.

Subjective tinnitus, which accounts for 90-95% of tinnitus cases[13] accompanied by auditory system damage, is referred to as "sensorineural" tinnitus or tinnitus of neurophysiologic origin[14-16]. The underlying neurophysiologic abnormalities believed to cause subjective tinnitus may be present throughout from the cochlea to the auditory cortex, although the majority of reported cases are driven by or associated with impairment of the cochlea [16,17]. Symptoms of subjective tinnitus characteristically includes a noise in the ears, such as ringing, hissing, buzzing, or whistling; the noise can be either an intermittent or continuous one. Due to its subjective nature, the characteristics of tinnitus are derived from patient reports.

Based on the temporal duration, tinnitus can be categorized as acute, subacute, or chronic. It is acute if the duration is shorter than three months and subacute until six months. If it lasts more than six months, it is considered chronic[18]. The temporal progress from the acute to the chronic condition is not entirely articultaed[13]. Subsequent references to tinnitus in this thesis focus on tinnitus of the chronic subjective type, which is the most common type of tinnitus by far [14].

2.2 Epidemiology of tinnitus

The studies of epidemiology of tinnitus are particularly difficult to be interpreted due to two reasons. Firstly, the definitions of this disorder are very diverse. Secondly, this subjective perception differs from patient to patient which makes measurement such as questionnaires very complicated. Tinnitus has become a major public health problem. It affects about 10% of population in Great Britain, 14.2% in Sweden, 8.4% in USA, 15.1% in Norway, 13% in Germany , 8% in France and 20.1% in Poland[19].

The incidence of tinnitus tends to increase with age, as approximately 18% of the population over 65 years of age experiences subjective tinnitus, after which the prevalence is either independent or declines slightly with age. Tinnitus was also reported in pediatric and adolescent patients, with the proportion of patients in this age group ranging from 7% to 34% [20]. The prevalence of tinnitus is significantly higher for males than females[21-24].

2.3 Hypothesized Origin of Tinnitus

Causes of tinnitus are mainly considered events, such as, impulse noise exposure which are associated with Tinnitus onset. Significant insults to the auditory periphery result in a loss of normal input to the auditory system and changes in numerous neurophysiological structures and neurochemicals; however, which of the structural and neurochemical changes is responsible for tinnitus is not known, even the triggering event is unequivocal. Pathological changes along the entire auditory pathway, can cause tinnitus[14,25]. Otologic disorders such as noise-induced hearing loss, presbycusis, otosclerosis, otitis, are considered the most common cause of tinnitus. The majority of tinnitus cases result from

the same conditions that give rise to hearing loss[26]. Eexcessive noise exposure among young adults and presbycusis among the elderly are frequent causes of tinnitus[14]. Other non-otologic tinnitus etiologies include cardiovascular and cerebrovascular disease, head/neck trauma and injury, and hyper- and hypothyroidism, multiple sclerosis, hyperlipidemia[27]. Furthermore, ototoxic medications, such as aspirin, naproxen and aminoglycosides (antibiotics such as gentamicin, streptomycin, or neomycin) and psychogenic factors such as depression, anxiety may damage outer hair cells triggering tinnitus[14,25,26]. However, a pure psychological origin of tinnitus is hotly debated.

Tinnitus is called idiopathic[28], if no clear associated event is identified with the tinnitus onset[14]. Normal audiogram does not necessitate any evidence of auditory damage. Because cochlear dead region and elevation of hearing thresholds in patients with normal audiogram in tinnitus frequency range have been observed[29,30]. In a separate research, it was reported that in tinnitus patients with standard audiogram, there is a significantly reduced amplitude of wave I of the evoked brain potential generated by the primary auditory nerve fibers. This provides physiological support for a "hidden hearing loss" that manifests as decreased neural output from the cochlea, leading to a re-normalization of neural brainstem response magnitude[31].

Indication of hearing loss in many of tinnitus patients could be observed using audiometric test suggesting that tinnitus is associated with different specific etiologies which affect a final common pathway, regardless of the level or pattern of damage in the auditory system[14,15,22]. However, some young patients with normal hearing range suffer from tinnitus while some older individuals with significant hearing loss do not have tinnitus[30,32-34]. However, in a comparison with their own control's groups (young participants without tinnitus and old individuals with tinnitus) revealed an interesting point. The groups with tinnitus showed hearing thresholds above 2 kHz ~10 dB higher, suggesting a relationship to audiometric function[29]. Overall, these findings suggest that tinnitus is generated by abnormal neural activity taking place anywhere in the auditory pathway.

2.4 Mechanisms of Tinnitus

Due to the heterogeneity of tinnitus patients[35] multiple mechanisms may be considered as possible explanations for tinnitus generation. The role of the cochlea is critical as a mechanism for tinnitus origin within the auditory brain. The determining of the physiological substrates underlying tinnitus is an essential component in the design of any effective therapies.

2.4.1 Discordant damage of inner hair cells (IHCs) and outer hair cells (OHCs) in Cochlea

Considering the cochlea as an isolated source of tinnitus from the rest of the auditory system is not adequate, but in some situations abnormal cochlear function can be considered as a tinnitus generator.

Cochlea impairment with ototoxic drugs or noise exposure starts with the outer hair cells (OHCs), and later affect inner hair cells (IHCs) if repeated[36]. In a partially affected organ of Corti, there might be three different OHCs and IHCs disorder-combinations. First, an area which OHCs and IHCs are affected, an area in which both are intact and an area which only OHCs are affected. Naturally the bottom of the tectorial membrane is not in a direct contact with top of the cilia of the IHCs and there is a small gap between them. The area with damaged OHCs and intact IHCs (the last category), this small gap might be also affected which tectorial membrane might directly move the cilia of the IHCs, thus causing the IHCs to depolarize[15]. Clinical support for such a modification in the auditory pathway leading to tinnitus perception has been reported, in that some tinnitus patients with high-frequency hearing loss their tinnitus frequency match to the point at which the loss begins[18,19].

Alternative mechanism proposed by LePage[22] et al considering those area in organ of Corti in which the basilar membrane has impaired OHCs ,but intact IHCs. OHCs regulate the sensitivity of IHCs by determining the activity level which the IHCs activity below this level interprets as no sound by brain. This level would not actually correlate to a zero-sound input, but a sound level considered as background activity. A loss of OHCs might

change that level causing a 'virtual' sound input, hence the normally imperceptible activity might be perceived as tinnitus.

2.4.2 Neurophysiological Model

Jastreboff's 'neurophysiological model'[37,38] includes classical auditory system pathways, limbic system, sympathetic autonomic nervous system and reticular formation (Figure 2-1). He further theorized that signal detection and classification networks play an important role in persistent tinnitus because neuronal networks adjust to the tinnitus signal, even if that signal is of low magnitude or is transitory. The cochlear induces feeble tinnitus activity in most people, but for many individuals, a habituation mechanism occurs after a short period of tinnitus initiation, so that the activity is no longer consciously perceived. Adverse emotional reinforcements, expressed as fear, anxiety, or tension, in turn lead to over-activation of the limbic and autonomic systems, which in turn leads to enhanced perceptual activity and persistence of perception. The distinctiveness between the perception of tinnitus on the one hand and the behavioral and emotional reaction to tinnitus on the other is explicitly stated in this model, as is the potentially for feedback loops between these distinct processes. The Jastreboff neurophysiological model suggests that, in tinnitus, the links between these elements of the central nervous system are governed by classical conditioning or associative learning[39]. Tinnitus Retraining Therapy which considers both aspects of the neurophysiological model, to facilitate habituation to the tinnitus signal and to reduce the impact of the reaction to that perception[38,40]. One possible criticism is that the model does not reflect the underlying complexity and dynamics of the human hearing system and is largely nonspecific.

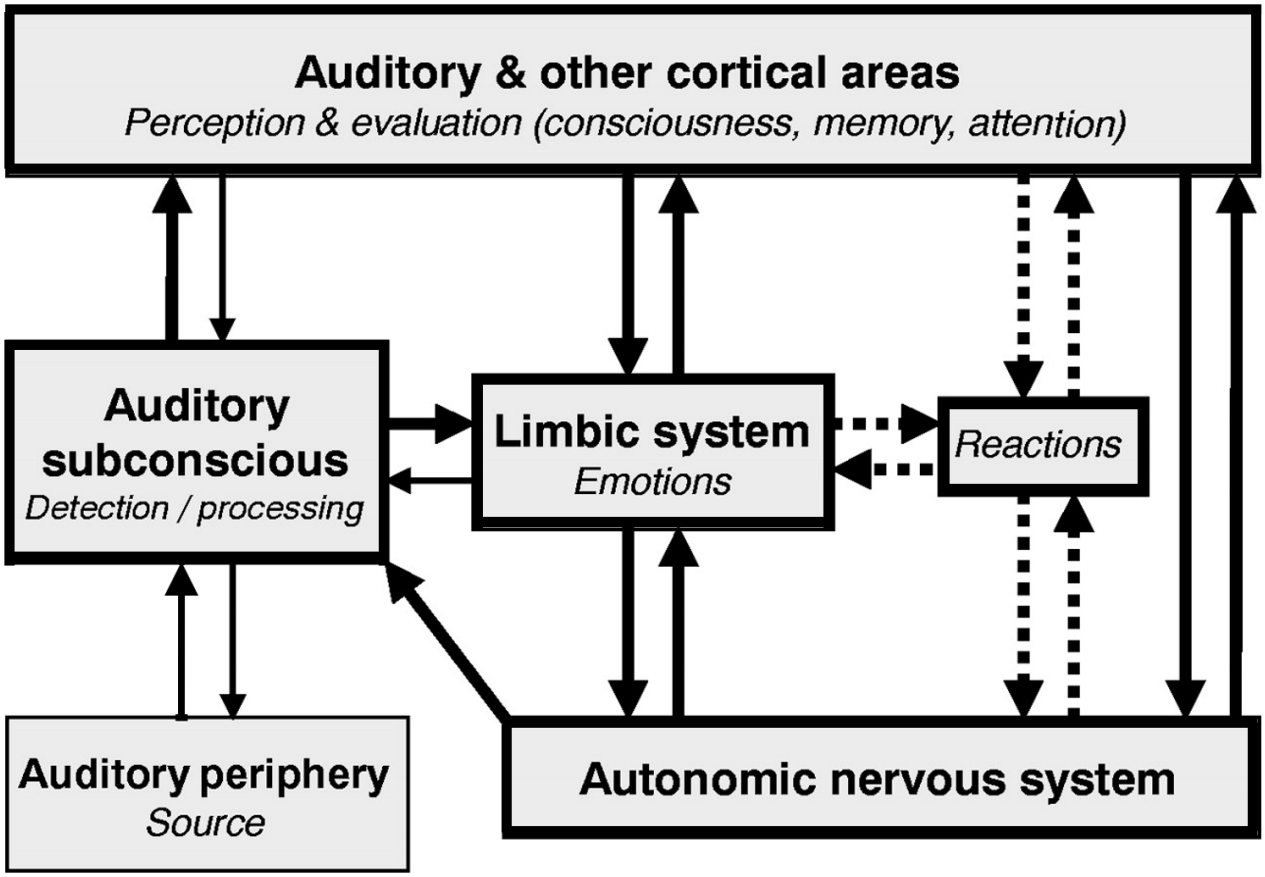

Figure 2-1: Diagrammatic representation of the Jastreboff neurophysiological model [41]. The thickness of arrows and boxes is an indication of how much strength has any system or the connection between two systems and in which direction this strength is higher. It should be emphasized that external auditory stimulus or tinnitus can be seen as a signal which evokes limbic and autonomic nervous systems, and consequently negative reactions .

2.4.3 Phantom Pain, Phantom Sound and Cortical Re-organization

The presence of the possibility of cortical re-organization in the auditory cortex after impairment of the auditory periphery, similar to that observed in phantom limb pain, was first studied in detail by Meikle[42]. The tonotopic pattern in the auditory cortex is altered upon deafferentation of a specific region of the cochlea, resulting in decreased activity in the cortical area within a corresponding characteristic frequency (CF). If the very same measurements are performed later, this region is still sound-sensitive, but many new neurons now have CFs adjacent to those of the "lesioned" region[43-46]. The re-organization impact on frequency mapping reveals an extended representation of frequencies at the

lesion edge, meaning that a high number of neurons become receptive to frequencies at the upper and lower edges of the hearing loss. It was suggested that spontaneous activity in these areas and the adjacent hypersensitive areas might be perceived as tinnitus[15]. Re-organization of the tonotopic map has been observed also in the part of auditory pathway like inferior colliculus of the chinchilla following a high-frequency, high intensity caused cochlear lesion[15]. Auditory periphery impairment results in an unbalance between excitatory and inhibitory inputs to auditory neurons at different stations of the hearing pathway. This indicates that neurons can adapt their homeostatic gain to retain normal auditory average firing rates after a reduction in excitatory input due to auditory deprivation. This process may lead to an enhancement of spontaneous auditory activity and / or to the enrollment of additional auditory areas.[47]

2.4.4 Contribution of the Somatosensory system

Tinnitus characteristics such as pitch and loudness can be modulated through teeth grinding, jaw clenching, shoulder or head movement. This relationship between somatosensory system and auditory system has raised the hypothesis that there is an interaction between auditory perception and somatosensory input[15]. One possible way to explain this is provided by experimental animal studies revealing that there is connectivity between the somatosensory system of the cervical spine and the temporomandibular region on the one hand, and the cochlear nuclei (CN) on the other hand[48,49]. The cell bodies of the afferent fibers of the cervical and temporomandibular somatosensory information are in the spinal ganglia. There is also the projection of some of these fibers to the central auditory system and more specifically to the dorsal CN[50]. The somatosensory projection to CN has a role in sound localization. There are some studies showing that this projection provides also somatosensory information for pursuing a sound source or for vocal production and speech development[51,52].

Stimulation of these somatosensory inputs can enhance or inhibit cells activation in the ventral and dorsal parts of the CN, leading to modulate their responses to sound, showing bimodal integration[53]. The changing in the somatosensory inputs in tinnitus patients could modulated intensity (increase and decrease) and pitch. But the modulation pattern are very variable among subjects and the changes are ephemeral[54]. The anatomical

connection between DCN and medullary somatosensory nuclei have been identified in animal studies , but a similar anatomical link in the human is not certain which can be regarded as a weak point of the hypothesis[15].

Chapter 3

3 Human Tinnitus

3.1 Human Tinnitus Studies vs. Animal Tinnitus Studies

Human tinnitus studies are able to explore perceptual and psychoacoustical characteristics of tinnitus, but direct access to the signal generating tinnitus anywhere in the auditory pathway using invasive methods, intracranial or intracerebral recording, is almost infeasible[14]. But non-invasive neuroimaging technics are applied such as MRI and fMRI[47,55-58], magnetoencephalography[59-61] and electroencephalography[62-64]. These techniques capture population level responses and particularly from the neocortex, which can be used to find alterations in neural networks that can be ascribed to tinnitus. The other non-invasive measures in human tinnitus studies are Auditory evoked potential (AEP)[65,66] and Auditory Brainstem Response (ABR)[67] which their characteristic such as latency and amplitude have shown abnormal activity associated with tinnitus. However, these techniques do not offer insight into the neural mechanism(s) giving rise to the phantom perception[65,68].

In animals' studies using local filed potential(LFPs), neural changes associated with tinnitus can be directly measured anywhere in auditory pathway. Neural correlates of tinnitus could be obtained using spontaneous firing rates or pair-wise spontaneous spike-firing correlations. But a description of tinnitus perceptual features (loudness, pitch, location) is difficult and it needs using time-consuming behavioral methods. Nonetheless, animal studies allow the direct investigation of neural changes associated with tinnitus. There are also many studies of the neural changes associated with hearing loss induced by different methods in animals, which are putative correlates of tinnitus[14,69]. As the main

investigation in this thesis is tinnitus in human subjects, therefore only human tinnitus studies would be reviewed in this chapter .

3.2 MRI and fMRI studies on Tinnitus

3.2.1 MRI studies on Tinnitus

Brain anatomical and functional alterations which might be correlated with tinnitus have been examined using neuroimaging technics such MRI or fMRI . Neuroplasticity and reorganization of the brain in many of the pathologies are considered as the source of anatomical and functional brain changes. Abnormal neuronal activity in tinnitus patients could be associated with changes in structure, gray and white matter volumes. The results of studies on structural changes in the brain of tinnitus patients are not consistent[47]. The reasons might be the heterogeneity of tinnitus characteristics, different brain morphometry techniques, such as voxel-based morphometry (VBM), surface-based morphometry (SBM), and the lack of meaningful tinnitus subtyping[47]. Adjamian et al. in a review article on the studies on structural changes of the brain in tinnitus patients (Table 3-1), showed inconsistencies among the results of these studies. To illustrate this, in one study Landgrebe et al. showed a significant reduction in gray matter in the inferior right colliculus and left hippocampus in the tinnitus group[70]. However, in this study no abnormalities were seen in the subcallosal area or in the thalamus as reported in previous published papers[71], have been observed. One of the confounding factors which influences the structural studies on tinnitus is hearing loss. The reason is brain structural changes might be the consequence of hearing loss and not specifically tinnitus. On the other hand, most of tinnitus patients to some degree have hearing loss. Hence, in a recent study, Allan et al. investigated anatomical changes associated with tinnitus in 128 study participants who were categorized into three distinct groups: Tinnitus with hearing loss, tinnitus with clinically normal hearing audiogram, and non-tinnitus controls with clinically normal hearing audiogram[72].Table.1 shows the result of this study for tinnitus patients with hearing loss which denoted by the letter k.

Table 3-1: Summary of main results of previous tinnitus voxel-based morphometry (VBM) and surface-based morphometry (SBM) studies compared to present results (table adapted from Adjamian et al., 2014)[47] . Present results are denoted by the letter K and are from tinnitus patients with hearing loss. Regular font letters denote VBM analyses and bold/italics letters SBM analyses. A = Mühlau et al. (*2006*); B = Landgrebe et al. (*2009*); C = Schneider et al. (*2009*); D = Husain et al. (*2011*); E = Leaver et al. (*2011*); F = Mahoney et al. (*2011*); G = Aldhafeeri et al. (*2012*); H = Leaver et al. (*2012*); I = Boyen et al. (*2013*); J = (Melcher et al. (*2013*); HL, Hearing loss; TIN, Tinnitus severity; vmPFC, ventromedial pre-frontal cortex; dmPFC, dorsomedial prefrontal Cortex.

	Group differences		Modulations	
Brain structure	Decreases	Increases	HL	TIN
Auditory gray matter				
Superior olivary complex	K			
Inferior colliculus	B	–	–	-
Medial geniculate body	FK	A	–	-
Heschl's gyrus (A1)	***CG***K*K*	I	***CG***K	I
Superior temporal gyrus (A2)	*GK*	DF	DI	H*H*
Non-auditory gray matter				
Ventromedial prefrontal cortex /subcallosal gyrus	AE*G*H*H*	(*K*)	IJK	*K*
Dorsomedial prefrontal cortex	*G*HH (*K*)	D	DIJ	-
Nucleus accumbens	–	–	K	-
Anterior cingulate	*G*	D	D	-
Posterior cingulate	*G*	–	J	-
Hippocampus	BI	–	I	-
Insula	–	–	–	H*H*
Supramarginal gyrus	H	–	I	H*H*
Occipito-parietal cortex	–	I	I	-
Orbito-frontal cortex	F	–	–	-
Superior frontal gyrus	(*K*)	–	–	-
Middle frontal gyrus	—	(*K*)	–	-
Middle temporal gyrus	–	I*K*	–	-
Precuneus	–	–	–	*K*
Fusiform gyrus	–	–	*K*	-

Allan et al. showed an increase in gray matter concentration in the superior olivary complex and a reduction in white matter in the medial geniculate nucleus for tinnitus participants. However surprisingly these effects have not been reported in the previous studies , although there are conflicting results such as changes in gray matter concentration in the medial geniculate body in tinnitus patients, which was reporting an increase in one study[71], and a decrease in the other study[73] compared to controls. At the the cortical level, both VBM and SBM analysis revealed a small reduction in gray matter thickness in the right Heschl's gyrus and a slightly larger reduction in cortical thickness

in the left auditory cortex (outside the Heschl's gyrus). However, outside the auditory structures, two significant clusters of tinnitus-related changes were detected in the same study: 1) a cortical thickness decrease in the left superior frontal gyrus 2) a cortical volume decrease with tinnitus severity in the right precuneus.

It should be emphasized that many significant findings of tinnitus reported in previous studies changes in cingulate cortex, hippocampus and insula, were not replicated by Allan et al. Even though they could replicate some effects from previous studies, the overall image shows non-replicability and contradictions. Allan et al. concluded that in the context of previous contradictory results, it is not yet possible with any confidence to associate tinnitus with anatomical changes in any specific parts of the brain[72].

3.2.2 fMRI studies of Tinnitus

Damage of cochlear hair cells is often considered as the source of subjective tinnitus[45]. But tinnitus with minor or no hearing loss in many studies has been observed[8]. Some studies also reported that in patients after cochlear tumor surgery where the eighth cranial nerve is sectioned, tinnitus still was reported in only 34% of the cases[74]. In the surgical treatment options for tinnitus, which includes destructive procedures, sectioning of the eighth cranial nerve in some tinnitus patients has been done. But 38-85% of the cases reported continuing tinnitus [57]. Even though the cochlea is disconnected from the brain in these patients, central mechanisms must be responsible for the tinnitus generation.

In the animal studies auditory pathway anywhere from cochlear to auditory cortex can be manipulated as an independent variable. From such experiments it was concluded that the firing pattern in the central auditory system was altered and could be a possible substrate of tinnitus. Noise trauma and ototoxic medications known to elicit tinnitus have been employed to induce tinnitus in experiments in animals. In induced animal tinnitus, alterations in spontaneous neuronal activation in several auditory brain centers were monitored, such as a reduction in spontaneous firing rates in the eighth cranial nerve and an enhancement of spontaneous firing rates at several levels in the auditory brainstem and cortex[75]. The findings of the animal research may lead us to conclude that bottom-up deafferentation causes a reduction in afferent signal input to the brainstem, which in turn

increases the amount of neuronal activity in the central auditory system, thereby causing tinnitus.

One of the neuroimaging technics which often is used to investigate changes in central auditory system is fMRI which measures changes in oxygenation level in blood flow and provides an indirect measure of neuronal activity[57].

In fMRI tinnitus studies several paradigms have been applied, 1) resting state compared to normal hearing control group 2) sound stimuli and measures of sound-evoked responses3) somatic modulation of tinnitus and compare the brain activity with no somatic modulation 4) rapid change of gaze or tonic lateral gaze causing or modulating tinnitus 5) pharmaceutical intervention using lidocaine leading to temporal change of the tinnitus 6) temporarily reduced tinnitus following the offset of an external acoustical stimulus[57]. In this thesis only resting state fMRI will be mentioned because other parameters do not seem to be relevant for our experimental approach.

3.2.2.1 Resting state

The findings of resting state fMRI in tinnitus patients reveals involvements of different brain regions.

3.2.2.1.1 Middle Temporal Gyrus

Middle Temporal Gyrus (MTG) which involves in sound recognition, semantic retrieval, semantic memory, language processing, showed increased spontaneous neural activity in the right side hemisphere in tinnitus patients[76]. Reduced functional connectivity between right MTG and the left thalamus has been observed, which was negatively correlated with Tinnitus Handicap Questionnaires (THQ) measuring tinnitus severity[77]. This finding could be explained based on the tinnitus model of Rauschecker et al[78]. Following this model, the tinnitus signal is normally abolished at the thalamic level (medial geniculate nucleus). This neutralization of the tinnitus signal occurs through an inhibitory feedback loop originating in paralimbic structures. The activity from paralimbic structures reaches the thalamic reticular nucleus, which in turn inhibits the medial geniculate nucleus resulting in no relay of tinnitus signal to higher level of auditory system. In tinnitus patients this inhibition mechanism could not occurs, because

the normal interaction between auditory system at the cortex level and at the thalamic level is compromised.

MTG is also considered within the Default Mode Network (DMN), referring to a connected cluster of brain structures that presumably includes the medial prefrontal cortex, posterior cingulate cortex, inferior parietal lobule, lateral temporal cortex, hippocampal formation, and precuneus. This brain network exhibits a lower activation level if a given subject is involved in a particular task, such as attentional or goal-directed behavior, but a higher activity level if the subject is awake and not engaged in a specified mental practice[79]. Phantom auditory sensation might involve engagement of the DMN. Several fMRI studies have found that human tinnitus distress is accompanied by enhanced resting-state functional connectivity within the default-mode network. Elevated resting-state connectivity patterns of the DMN may serve a pivotal role in neuropathological hallmarks underlying chronic tinnitus[80,81].

3.2.2.1.2 Frontal Cortex

The superior frontal gyrus (SFG) and inferior frontal gyrus (IFG) showed increased neural activity in tinnitus patients compared to healthy controls. Abnormal activity of frontal cortex which has been observed in resting-state fMRI studies could be a direct mechanism which leads a transition from acute to chronic tinnitus[76,82]. Chen et al. showed that, the SFG could be regarded as a main cortical hub affected by tinnitus. SFG can receive and integrate sensory and emotional aspects of tinnitus. A presence of tinnitus signal in auditory cortex increased attentional processing which manifests itself as an enhanced activity in superior temporal gyrus[83].

The IFG is a region of response inhibition which is postulated to be the underlying mechanism by which the PFC exercises its modulatory effects on both subcortical and posterior cortical regions to achieve executional control. In neuroimaging studies on response inhibition such as Go/No Go task, consistently and especially were demonstrated that right-lateralized inferior frontal cortex (IFC) region is activated, leading to perform the response inhibition[84]. In tinnitus patients IFG activity might reflect

the IFG " attempts" to regulate the bottom-up attention appropriation to the tinnitus percept in a top-down manner[85].

In a fMRI study of the Stroop performance task, tinnitus patients demonstrated elevated activation in the dorsolateral prefrontal cortex (dlPFC), cingulate gyrus, and ventromedial prefrontal cortex (vmPFC) compared with controls. The other finding of this study was a positive correlation between reduced executive functions in prefrontal cortex activity in tinnitus patients. These findings suggest that a lack of inhibitory modulation due to disturbed top-down cognitive control may sustain tinnitus by impeding mechanisms of habituation. This executive function deficit, caused by changes in the prefrontal cortex, could be a key contributor to the development and persistence of tinnitus[78,86,87].

3.2.2.1.3 Limbic system

Functional connectivity between insula and auditory cortex in tinnitus patients has been investigated in many fMRI studies. Increased spontaneous neuronal activity and functional connectivity with auditory cortex of the bilateral anterior insula were shown in resting-state fMRI[76,81]. In another study using fMRI in tinnitus increased connectivity between the auditory cortices and the amygdala in the tinnitus compared to age-matched normal hearing controls[88]was observed. This association between auditory and limbic regions has been proposed by brain imaging studies and conceptual models of tinnitus. Jastreboff (1990) in his neurophysiological model of tinnitus describes the interaction between the limbic and auditory systems. The model emphasizes the role of habituation to the tinnitus percept allowing a patient to ignore the phantom sound. However, when "negative reinforcement" is present, the limbic system can cause the auditory activity to be perceived, which could then lead to a vicious feedback loop. The correlation between the auditory resting state network and limbic areas fits the framework of this hypothesis. The relationship between tinnitus and emotional processing in an emotional sentences task was examined, revealing changes in activation in limbic and frontal areas in highly distressed tinnitus patients. They suggest that the significant regions, including the anterior cingulate cortex, the medial cingulate cortex, the insula and the precuneus, are part of a general distress network and are not specific to tinnitus[89]. The exact mechanism of how these networks are involved in tinnitus distress remain unclear.

2.1.3 EEG and MEG Studies of Tinnitus

In animal studies have been shown that deprivation of the afferent input gives rise to reduced inhibition, which then causes the observable hyper-excitability in regions of the central auditory system, as reflected by an elevated spontaneous neuronal firing rate in cortical and subcortical auditory structures[90]. Activity in the auditory cortex, which corresponds with tinnitus frequency, has also been demonstrated to be increased and associated with perceptual tinnitus intrusiveness in human magnetoencephalography (MEG) studies [91].

Using MEG also has showed changes of the tonotopic map in human tinnitus sufferers[92]. Deprived cortical neurons begin to respond to activities of their neighboring cells via lateral connections leading to an overrepresentation and overactivation of the edge frequencies and maladaptive auditory cortex reorganization[45]. The latter has been demonstrated by MEG studies yielding a positive correlation of the amount of "edge" distortion and perceived tinnitus strength[93].

In humans only indirect assessments of neuronal synchronization in tinnitus patients exist i.e., by means of EEG and MEG. Comparing resting MEG power spectra, it has been observed markedly reduced alpha (8-12Hz) and increased delta (1-4Hz) power in awake tinnitus patients relative to normal hearing controls[94]. The differences were most pronounced over perisylvian regions and were significantly correlated to tinnitus-related distress.

These observations, especially the delta increase, stimulated the idea that chronic tinnitus may not just result from unleashed increased neuronal firing but from activities in a 'distributed network of neurons in different brain regions including auditory and non-auditory areas that process basic (phantom) sound sensation and related affective and motivational aspects. It has been shown that slow frequency oscillations in the delta/theta range seem to modulate (control) neuronal spike synchronization and its concomitant activity in the gamma range[95,96].

In tinnitus sufferers and normal hearing subjects changes in brain oscillations have been shown: (1) the slow-wave activity(2-7Hz) was strongly correlated with activities in the gamma frequency range (50 - 60Hz), (2) the activity in the gamma band was enhanced in individuals suffering from tinnitus, and (3) the ~ 55Hz activity was significantly

associated to the laterality of the tinnitus percept[60]. These results, together with the observed peripheral damage of the included patients' auditory systems led to the conclusion that 'deafferentation' result in corticothalamic and corticolimbic interplay leading to enhanced slow oscillatory activity of these cell assemblies. Slow-wave activity sustains high-frequency oscillations (perhaps indirectly by release of inhibition, normally sustained by the alpha rhythm) reflecting spatially restricted synchronized firing of neurons underlying the tinnitus percept'. These findings and notions from animal studies funneled into the so called 'Synchronization by Loss of Inhibition Model (SLIM)'[97].
Nevertheless, as previously theorized by De Ridder et al. (2011), such high-frequency gamma band activation in sensory cortex only yields "conscious perception" if it is coupled with larger coactivated "(self-)awareness" and "salience" brain networks[98]. Learning mechanisms may link phantom awareness to distress, which emerges as a simultaneously co-activated emotion-distress network consisting of the anterior cingulate cortex, anterior insula, and amygdala. Memory mechanisms are known to take a role in the persistence of "phantom awareness" consciousness, i.e., in its chronification. This process was also observable with the analysis of source-localized EEG recordings of tinnitus patients indicating that the generators involved in the acute/sub-acute state changed over time with increased activity in auditory but also non-auditory areas such as the anterior cingulate cortex, dorsal lateral prefrontal cortex, insula, supplementary motor area, orbitofrontal cortex, parahippocampus, posterior cingulate cortex and the precuneus[99,100].

3.2.3 Neurofeedback and neuroplasticity

Neuroplasticity is the ability of brain to form a new neural connections and pathways as a result of our interaction with environment, training or practice, through the whole life. Neurofeedback is a scientifically recognized method in which patients learn to control electronically/metabolically recorded brain activity themselves and thereby specifically activate or relax the brain. Neurofeedback is based on the brain's plasticity. In the neurofeedback procedures continuous information of brain activity is provided to the subject, humans or animals, permitting a voluntary regulation of that activity through feedback[101,102]. Different neuroimaging technics have been implemented for

neurofeedback. Electro-encephalography (EEG), and electro-physiology use neuroelectric activity[103-106], magneto- encephalography (MEG) use magnetic fields produced by electrical currents in the brain[107], and functional near-infrared spectroscopy (fNIRS)[108,109] and real time functional magnetic resonance imaging (rtfMRI)[110,111] by which caused by metabolic activity of the brain.

The first study on neurofeedback in human subject was performed in 1969 using alpha waves (8-12 Hz) [112]. The promising results of this study leads to other work, where the researchers demonstrated that animals are also able to modulate their brain activity by providing them feedback[104]. However, the clinical application of neurofeedback took many years. It has been shown that training of self-regulation of slow cortical potentials (SCPs) in patient with drug-resistant epilepsy leads to less experienced frequent ictal events[113]. In a neurofeedback study child with attention deficit–hyperactivity disorder (ADHD) could learn to regulate negative SCPs. This well-controlled study resulted in significant improvement in behavior, attention of ADHD child[114].

3.2.4 Neurofeedback in chronic tinnitus

Loss of inhibition seems to be an initial key process in the chain of reactions that leads to the phantom perception of tinnitus and therefore appears to be the point where specific research and non-invasive therapeutic intervention may be applied[94]. Auditory cortex alpha activity i.e., the EEG-alpha frequency component generated in the auditory cortex, becomes central, since alpha activity in sensory cortices has been shown to be an indicator of ongoing inhibition and of the balance of excitation and inhibition in a cortical structure[115,116]. This notion finds additional support by the observation of an inverse relation of auditory cortex alpha and gamma activity in control subjects and tinnitus sufferers with the latter showing a somewhat steeper regression line[97]. However, most of the research on alpha EEG was carried out in the visual and somato-sensory/motor domain and therefore, it was debated whether results apply also to the auditory modality, even the existence of an auditory cortex alpha was questioned. In an excellent review article Weisz et al. argue convincingly that both an auditory cortex alpha exists and that it signals the same functional state as other sensory structures[117]. However, the small volume of the primary auditory cortex results in alpha superposition by stronger signals

from visual and somato-sensory/motor structures and therefore is difficult to isolate. However, this signal can be picked up and used for neurofeedback as demonstrated in the already mentioned MEG study by Weisz et al[94].

The clear spectral differences observed suggested the application of neurofeedback, a procedure that has proven to enable normalization of EEG spectra in various applications. Neurofeedback training enables directed changes of unperceivable neurophysiological processes via sensory feedback-information about these processes and operant learning. One has to keep in mind, however, that the specificity of changes achieved by this method depends on the specificity the regulated neurophysiological process has been captured - and with tinnitus primarily activity localized in the auditory system and its maladjusted connections are relevant.

Alpha-activity feedback is an option to stimulate reestablishing inhibition in the affected part of the auditory cortex and possibly reversing maladaptive cortical reorganization. And indeed, some progress has been made already. With the aim of pinpointing the activity of frontal and temporal regions alpha/delta ratio neurofeedback applied to 21 tinnitus sufferers using signals from 4 front-central electrodes (F3, F4, FC1, and FC2)[59]. Participants were successful in changing their EEG spectra and their tinnitus intensity became reduced (~ 20% reduction of tinnitus distress). The same group improved experimental methods extending the number of EEG channels to 32 and source montages were implemented to record signals from more circumscribed areas[117]. Also, the feedback was made 2-dimensional, so that both changes in alpha as well as delta could be pursued synchronously but independently[118]. Two experiments were conducted, one with alpha enhancement – delta suppression trained using the 2D feedback (‘Alpha-Delta Training’), and a second where sounds of 5 s duration were presented and participants had to increase alpha as much as possible during stimulation (called ‘Desynchronization Suppression Training’). Interestingly, in both experiments’ delta didn’t change much. Participants, however, were quite successful in alpha enhancement in both experiments and reached a tinnitus distress reduction of 28% and 25%, respectively[119].

In another study ‘source projection to two temporal sources’ based on 29 EEG electrodes was used. According to this procedure the influence of alpha activity of neighboring cortical structures, however, may still not have been sufficiently excluded and this way the efficacy of neurofeedback became possibly reduced[119].

Another technique which is called 'EEG Finger-Print (EFP)' was proposed by group in Tel Aviv[120]. To put it simply, this method can be seen as a fMRI calibrated filter based on the varying spectral characteristics of the best predicting single EEG-signal across a predefined time window. Once calibrated for a particular (cortical or subcortical) brain region using EEG-fMRI parallel recordings, this 'filter' can be used with the same subject to extract the activity in this particular region based just on EEG recordings in subsequent sessions. Although this method appears to be promising further neurophysiological studies are necessary to validate the hypothesis of EEG recordings from subcortical sources. The use of the EEG-Finger-Print for neurofeedback at present is also economically and clinically too expensive and too MR-time consuming.

3.2.5 EEG neurofeedback in electrode level vs source level

In order to learn to permanently increase local alpha activity, and hence the inhibitory potential, a neurofeedback learning procedure is a promising non-invasive strategy. However, the unspecificity of the target variable may constitute one of the major obstacles for consistent perceptual changes following alpha increase neurofeedback training. This is because the human waking EEG is dominated by alpha activity from the posterior visual system and, in states of motor quiescence, by the sensorimotor alpha rhythm. The sensorimotor alpha originates in the fronto-central areas and "overrides" the alpha from the auditory cortex which projects mainly from a tangentially oriented temporal dipole to central areas at the midline. A successful neurofeedback training procedure therefore requires specific control of alpha activity from the auditory cortex with simultaneous suppression of the visual and somatosensory alpha activity.

An alpha neurofeedback system for tinnitus patients has faced two problems. The first is how to derive EEG alpha oscillations in an on-line paradigm from the auditory cortex. Using EEG recording data from even high-density EEG does not automatically ensure that ongoing oscillatory activity from the auditory cortex can be recorded or trained. This problem originates from the general phenomena of volume conduction. Volume conduction is the cause for the dissipation of the electrical brain activity to sensors at different positions. In other words, the recording EEG electrodes are not in direct contact

with the neurons; there are different tissue layers including cortico-spinal fluid, skull and scalp separating the two and smearing the local activity of a neural generator on the head surface [121]. The volume conduction effect leads to mixed activity of several simultaneously active sources recorded at individual sensors with EEG. This effect can lead to unreliable interpretations of the recorded EEG signal due to an unknown number of different, distant, neural sources [122,123]. To reconstruct the sources from the recorded EEG data, which is referred to as "inverse problem", a number of methods have been proposed with which the location of the sources of the EEG can be computed from measurements at the scalp. The inverse problem in the EEG is known as an ill-posed problem [124], because a solution for the inverse problem can be found only by introducing constraints and making assumptions that may be incomplete. The more physiologically appropriate these assumptions are, the more reliable are the source estimations [125]. Many techniques have been developed to overcome the inverse problem, allowing a spatial resolution of centimeters on the scalp compared to a millimeter scale on the cortex [122,125,126]. Some of the existing methods for solving the EEG inverse problem are beamforming methods such as dynamic imaging of coherent sources [127], source space-based distributed and sparse methods (sLoreta) [128]. Using the aforementioned source localization methods in real-time procedures for EEG neurofeedback of auditory cortex, allows to give a patient feedback directly from the ongoing activity in auditory sources. There are, however, three main issues with regard to the usage of these techniques in online procedures: the low signal-to-noise ratio (SNR), the limited time available for computations and the computation cost. We therefore propose a new method that does not encounter these three limitations. The second problem is auditory cortex alpha overlapping with alpha from neighboring sensory cortices, particularly visual and somatosensory/motor structures. The contribution of non-auditory alpha, in particular alpha originating from occipital cortex to the standard alpha neurofeedback procedure, is therefore important.

Chapter 4

II. Experimental Part

4 Real-Time monitoring of auditory cortex activity

To overcome the two mentioned problems in the last chapter, we propose a novel method and a new experimental design for monitoring of auditory alpha activity. The method proposed consists of extracting a pattern of EEG data from recording during stimulation of primary auditory cortex before alpha neurofeedback sessions for each patient separately. This EEG- pattern supposedly stemming from auditory activity would then be compared in real-time with the ongoing alpha EEG oscillation during neurofeedback sessions resulting in an index of how much these two ongoing spontaneous and auditory-stimulation-based patterns would be similar. The simulated brain pattern functions like a spatial filter and corrects for the volume conduction effect and can be used to reinforce alpha activity originating from the primary auditory cortex. As this similarity-based spatial filter detects the activity of the auditory cortex on the EEG electrode level not in the source space, we need to specify the specific channels that reflect auditory cortex activity and from where at the scalp the feedback is provided to the patient. In tinnitus EEG neurofeedback studies, fronto-central channels [129] were generally selected due to the dipole-orientation of auditory sources. However, the selection of the specific channels presumably projecting from the auditory cortex does not necessarily reflect the patient's auditory alpha if the volume conduction phenomenon is not considered. We propose a novel method for locating those specific EEG channels for neurofeedback. For this purpose, we have designed a localizer procedure with two aims; to find the specific EEG

channels for real-time feedback and to design a spatial filter to detect auditory alpha for each patient individually. Furthermore, the usage of multi-sensory stimulation in addition might make it possible to suppress visual and somatosensory alpha during learning of alpha neurofeedback control, and it is well documented that sensory input leads to modality-specific suppression of alpha activity at cortical regions involved in processing such input [130], thereby facilitating the appearance and recording of alpha activity from the auditory cortex.

4.1 Method

4.1.1 Participant recruitment

Three male patients with chronic tinnitus were recruited for this phase of the study. Table 4-1 summarizes the characteristics of the three patients. All three patients provided written informed consent after receiving a detailed explanation of the experimental procedures. The study is in full compliance with the ethical practice of Medical Faculty of the University of Tubingen. Informed consent approved by Internal Review Board of the Medical Faculty of the University of Tubingen is in accordance with the research protocol, following the principles as laid down in the current version of the Declaration of Helsinki.

Table 4-1: Characteristics of tinnitus patients

Number	Age	One or both side	Tinnitus duration	THI
1	43	both	1.5	38
2	54	both	2	72
3	52	both	2.5	78

THI: Tinnitus Handicap Inventory

4.1.2 Material

The actiCAP with its 64 active electrodes (based on high-quality Ag/AgCl electrodes) was placed with a standard EEG-cap using the Brain Products recording system (BrainAmp). Two reference electrodes were attached on both mastoids. In order to

monitor vertical eye-movements, two electrodes were placed infra-orbital and supra-orbital to the right eye. One EEG electrode was placed 1cm lateral to the left outer canthus. The air compressor for producing air flow was Schneider Compressor SEM 30-8-4 W DGKA333005[131]. The pressure of the air flow were controlled with two systems, the compressor pressures control valve and the other valve which was installed on the tube which carries the air flow from the compressor to the chin of the patient (Figure 4-1).

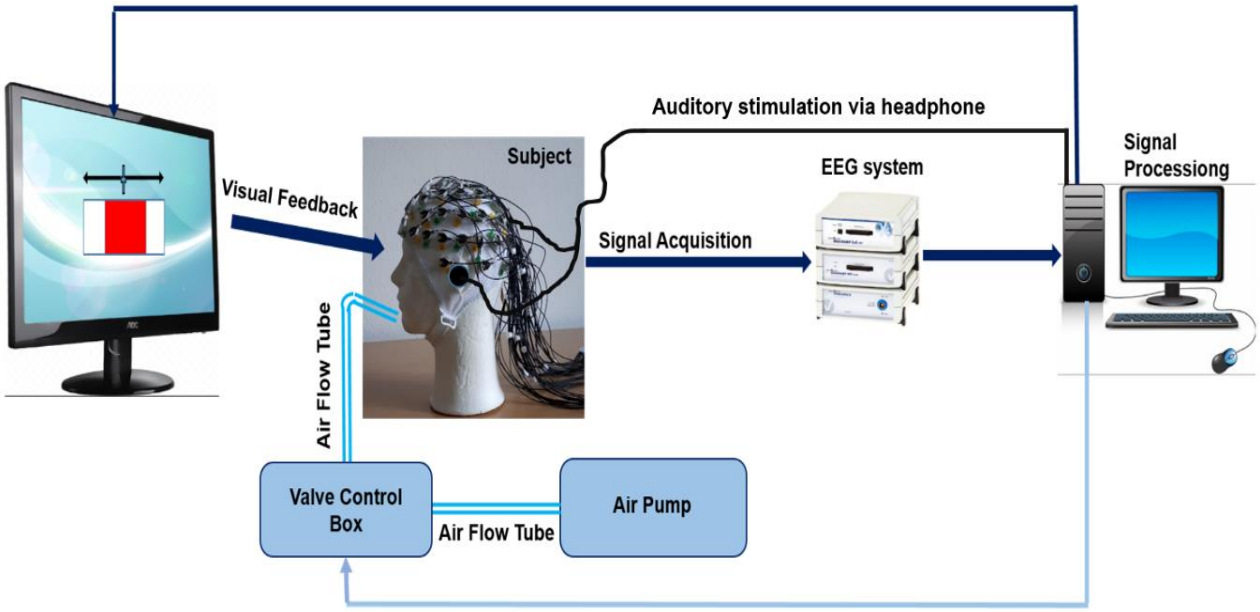

Figure 4-1: Experimental situation. Neurofeedback setup including EEG, an air-pump for the tactile stimulation of the mental protuberance, auditory stimulation via earphones and hardware for online EEG signal processing.

The amplitude-modulated auditory stimulation with 40 Hz modulation frequency and 1000 Hz carrier frequency (Figure 4-2) activates the primary auditory cortex in particular [132,133]. The topography of 40 Hz is a map of the brain activity that depicts the activity of the auditory cortices. This map can therefore be used to determine to what extent the brain activity in a specific time window resembles auditory cortex activity. We used this map as a spatial filter, which stresses the activity from the auditory areas. In real-time, the topography of each frequency in alpha band (8-13 with 1 Hz resolution) has to be compared to the topography of 40 Hz which results in a similarity index. On the one hand the similarity index reflects how active non-auditory alpha sources are. This means that

an increase in the similarity index would be accompanied by less presence of non-auditory alpha sources, yet it does not reflect the amplitude of auditory sources. On the other hand, the alpha power from the optimal channels is a representation of the summation of the amplitude of alpha sources. Therefore, the product of similarity index and alpha power of the optimal channels indicates how much alpha auditory source is contributing to the alpha power on the electrode level.

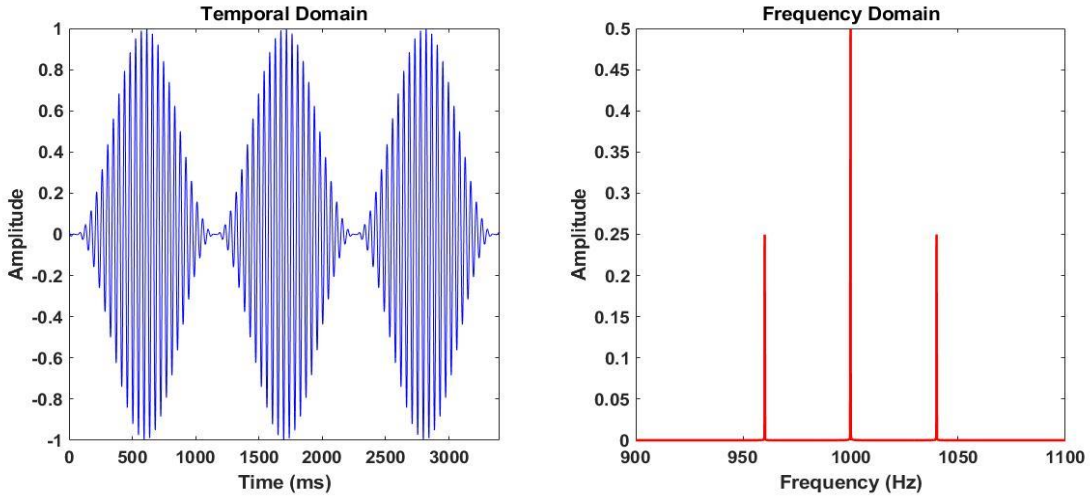

Figure 4-2: Amplitude modulated signal (time domain) and frequency domain. Its primary energy is at the carrier frequency (1000 Hz) and two sidebands of energy are located at the CF-MF (960 Hz) and CF+MF (1040 Hz).

4.1.3 Design and experimental procedure

The similarity method consists of extracting and deriving a pattern of EEG data from a stimulation of the primary auditory cortex before neurofeedback sessions for each patient separately. This pattern will be compared in real-time with the ongoing alpha EEG oscillation during the neurofeedback sessions. The similarity method calculates online how much alpha oscillation from auditory cortex contributes to real-time neurofeedback recorded alpha rhythm at the electrode level. For this purpose, we employ localizer runs before sessions for two reasons; the first is to obtain an individual brain pattern when the primary auditory cortices are activated via specific auditory stimulation. Secondly, to specify the EEG channels (optimal channels) for which the auditory cortex activity

contributes more to the recorded electrical activity than the other EEG channels. The feedback presented to the patient is a multiplication of alpha power in optimal channels with the similarity index, which is the final outcome of the similarity method (see below).

4.1.3.1 Eye Movements Calibration and Localizer Runs Procedure

The eye movement calibration run consisted of two phases; a horizontal eye movement phase in which the patient had to look at the left and right part of the screen 10 times (5 times left and 5 times right), and a vertical eye movement phase in which the patient had to look at the upper and lower part of the screen 10 times (5 times up and 5 times down). The aim of eye movement calibration run is to specify a threshold for horizontal and vertical eye movements for online and offline rejection of EEG data. We employed four localizer runs in order to localize the auditory cortex sources separately for each patient. Each of the runs 1, 2, 3, and 4 included 100 trials which lasted 1 sec followed by 2 sec of inter-trial interval. During a single run, patients were instructed to focus on a black cross in the center of a gray screen. In the first and third runs, *amplitude modulated auditory stimulation* was presented in each trial, with the patients' eyes open in the first run and closed in the third run. Participants were permitted to blink every other trial after the offset of the auditory stimulation, which was cued by a "green cross" (at the center of the screen on top of the black cross) for 2 sec. In the second run, the patients were instructed to look at the black cross at the center of the screen while no auditory stimulation was presented. Again, blinking was allowed only when the green cross appeared. In the final run, they had to simply close their eyes upon the experimenter's cue.

4.1.3.2 Neurofeedback Procedure

All three patients participated in 3 experimental visits, each lasting for approximately 2 hours, including preparation. The number and locations of the EEG channels were identical to those in the localizer runs. Each run, composed of 20 trials, began with a 5 sec rest period (black cross on a gray background), followed by a 10 sec non-regulation phase, which was cued by a green fixation cross in the center of the screen. There were two reasons for implementing the non-regulation phase. Firstly, it gave time the patient to blink or move the head if necessary. Secondly, in the "non-regulation" phase the patient was presented with the specific auditory stimulus (pink noise) which gives the patient a

relief of tinnitus through masking the tinnitus during non-regulation phase [134]. It also suppresses the alpha oscillation in the auditory cortex during its presentation, which could lead to a rebound of alpha oscillation in the regulation phase. This rebound can exceed the pre-stimulus baseline level of alpha oscillation [135] facilitating alpha upregulation in the regulation phase.

The non-regulation phase was followed by 12 sec of regulation, cued by a black horizontal arrow pointing in both opposite horizontal directions. A red horizontal "thermometer" was extended/shortened from the center of the screen, depending on the amount of alpha activity from auditory cortex (Figure 4-3).

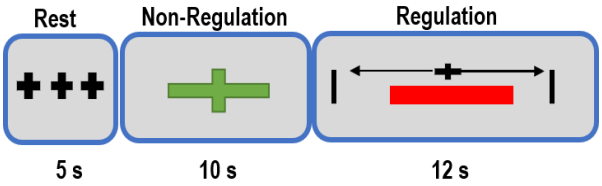

Figure 4-3: Experimental protocol. The patient receives 20 trials per run. Each run starts with 5 sec rest (black cross on a gray background). It is followed by 10 sec of non-regulation phase which is cued by a green fixation cross in the center. During this phase, pink noise is presented to the patient and he/she is allowed to blink or to move his/her head. Then, there is a 12 sec regulation phase, cued by two black horizontal arrows. During this condition, a red horizontal thermometer will be extended/shortened from the center of the screen based on the amount of the alpha activity coming from the auditory cortex. In addition, during rest and regulation phases, a tactile stimulation consisting of airflow with constant velocity stimulates the mental protuberance.

4.1.4 Analysis and statistics

4.1.4.1 Offline

The analysis of localizer runs entails the following procedure: the EEG data was firstly re-referenced to the average of both mastoids. The mastoids were chosen as reference electrodes because, despite being close to all other electrodes, they receive little signals from the brain. In addition, since we anticipated that the main effects would be on the central channels due to the orientation of the dipole sources of the primary auditory cortex, the EEG data were re-referenced to the mastoids channels to recapture the fronto-central activity. Horizontal and vertical eye movement signals in each trial (1 sec) were

calculated and each value in each time point of these signals was compared to the Absolute Maximum Values (AMV) of eye movement calibration runs for both vertical and horizontal eye movements. If 0.3 portion of vertical or horizontal eye movement signal was larger than AMV, the trial was rejected.

Fourier transform with 1 Hz resolution was applied to determine the frequency in alpha (8-13Hz) band in all localizer runs that had maximum power per one Hz (Frequency of Maximum Power (FMP)). Since the amplitude-modulated sound occurred at 40 Hz, the Fourier transform was applied to extract real and imaginary parts of 40 Hz as well as in FMP for each trial of each localizer run. To select optimal channels reflecting auditory cortex activity, we used singular value decomposition (SVD) in two steps.
Singular value decomposition produces a diagonal matrix s of the same dimension as X (m-by-n) of which the row of X are observations and columns of X are variables and s are non-negative diagonal elements in decreasing order, and unitary matrices u and v such that

$$[u, s, v] = svd(X) \tag{4.1}$$

$$X = u * s * v^T \tag{4.2}$$

$$U = u * s \tag{4.3}$$

$$P = s * v \tag{4.4}$$

The columns of u and the columns of v are called the left-singular vectors and the right-singular vectors of X respectively and s stands for singular values and v denotes the relation between pattern and principal components. P stands for pattern which, in our case, is the Auditory Steady State Response (ASSR) pattern as brain response to the auditory stimulation or FMP pattern. U can demonstrate which patterns can be selected as auditory and occipital patterns.

Firstly, for each trial of each localizer, we applied SVD on real and imaginary parts on FMP and 40 Hz separately to calculate two different topographies of the brain signal in each trial. The first column of *u* in equation (4.1) for 40 Hz is the topography of 40 Hz and the first column of *u* for FMP is the topography of FMP. The reason behind applying SVD on the trial data, and not the averaged data, is because the phase of FMP across trials is not constant. At this level, the observations are channels (61 EEG electrodes after

excluding EOG electrodes) and variables are real and imaginary parts of 40 Hz or FMP. The aim of this transformation is to gain both real and imaginary information simultaneously for one topography. Since we have four localizer runs, each consisting of 100 trials, as well as two topographies for each trial, in 40 Hz and in FMP, therefore we have a total of 800 topographies for all localizer runs. Secondly, we again applied SVD to these 800 topographies. At this level, the input for SVD is a matrix with 800 observations (rows) which represent the topography in 40 Hz and topography in FMP for all trials of localizer runs (1, 2, 3, and 4) respectively and variables (columns) are 61 EEG channels. U was calculated on the basis of equation (4.3).

The selection of channels for auditory and occipital areas was realized as follows:

1. As the number of trials for each localizer is 100, U is averaged in the first dimension with 100 row steps. This resulted in a new matrix called U_F with 8 rows and 61 columns which 8 rows corresponding to the FMP and the 40 Hz topography for each localizer run respectively. The columns are the components.
2. We plotted the cumulative sum of singular values (in descending order) with each value divided by the total sum of singular values prior to plotting. The plot will show the fraction of total variance retained vs. the number of singular values. We preserved components which have around 98.8% or 99% of the total variance of the data.
3. In the component space (U_F), the component which reflects auditory activity has the larger absolute value in the first and fifths rows of U_F than in the second row and sixths rows of U_F respectively. Moreover, it has the smaller absolute value in the third and seventh rows than in the fourth and eight rows in the component space (U_F) respectively. The reason for this selection is the presentation of auditory stimulus in localizer run 1 and 3 where the ASSR has more weight than occipital alpha.
4. In the component space (U_F), the component that reflects occipital activity is exactly the reverse. This selection was chosen on account of the absence of the auditory stimulus in localizer 2 and 4 in which occipital alpha is dominant over 40 Hz brain activity.
5. Following selection of auditory and occipital components, we selected the

singular values corresponding to that component and computed the pattern of ASSR and occipital alpha (equation (4.4)).

6. We plotted these two patterns.
7. The channels that reflect auditory activity are those with the highest values in auditory pattern and the lowest values in occipital pattern (minimal alpha).
8. The channels that reflect occipital activity are those channels with the maximum value in occipital pattern and the minimum value in an auditory pattern (maximal alpha).

4.1.4.2 Online

The segment of data for real-time analysis was 1 second and the overlap between two segments was 500 milliseconds. The same similarity method was applied in the feedback training phase and the rest period. Online individual normalization during regulation phase for each segment of the data (1 sec) was carried out such that the average of output indices (TFP) for rest periods (5 sec) was calculated, regulation phase output index (TFP) for each segment of the data (1 sec) was then subtracted from the averaged rest and, finally, also divided by the averaged rest value. This is the final total filtered power (FTFP) which the patient perceives as a red horizontal thermometer. During the "non-regulation" phase, the patients are permitted to blink. However, if the patient blinks during the other intervals and the rejection criteria – which is the same as in localizer runs procedures – is met, the feedback value is not updated for that time interval. During the "regulation" phase, participants were instructed to self-up-regulate the alpha activity. No specific instruction for eventual cognitive strategies was given. If patient asked, he/she was told "to try his/her own strategy, everybody has his/her own strategy". Additionally, during the rest and regulation phase, we presented a tactile stimulation which consisted of an air flow with constant velocity that stimulated the mental protuberance to block sensory motor alpha-rhythm (SMR). The stimulation of mental protuberance suppresses the alpha activity in somatosensory regions adjacent to the auditory cortex. We hypothesize that suppression of somatosensory alpha through tactile stimulation and

suppression of occipital visual alpha from the visual stimulation of the visual feedback signal at the screen facilitates auditory alpha self-regulation.

As mentioned above, the aim of the localizer runs is to provide two kinds of information. They show us the brain pattern when the brain is stimulated with specific auditory stimulation and select those channels which supposedly reflect auditory cortex activity.

We selected 1 Hz resolution for less computational time in online processing. The segment of data for real-time analysis was 1 second and the overlap of the window between two segments was 500 milliseconds. The real-time calculation for each segment of data (1 sec) followed these steps:

1. Absolute maximum values of the horizontal and vertical eye movements for online rejection were extracted from the eye calibration runs.
2. The 61 EEG channels (EOG channels excluded) were divided into left and right hemispheres. The midline EEG channels were assigned to both the left and right hemispheres to avoid any lateralization problems. This separation was proceeded because the auditory stimulus which we present to a patient in the localizer 1 activates the left and right auditory sources combined. Under this condition, the left and the right auditory sources are highly correlated [133,136,137]. Furthermore, the spontaneous alpha oscillations in left and right auditory cortices are not correlated. Thus, we calculated the real-time auditory EEG for each hemisphere separately, and the final output feedback consisted of the sum of the left and right hemispheres.
3. The imaginary and real parts of the Fourier Transformation at 40 Hz for 61 EEG channels were extracted from the first localizer run. We then applied SVD on the real and imaginary parts on the left and right hemispheres channels separately. The first column of u in equation (4.1) for the right hemisphere was the 40 Hz topography of this hemisphere. Likewise, the first column of u in equation (4.1) for the left hemisphere was the left hemisphere topography of 40 Hz.
4. Each segment of data (1 sec) was examined to ascertain whether it contained

EOG artifacts or not in the same manner as for the localizer runs. If recorded data did not contain EOG artifact, the calculation continued.

5. The cleaned segment of the data was decomposed with Fourier Transformation, giving real and imaginary parts for 8, 9, 10, 11, 12, and 13 Hz. We then applied SVD on real and imaginary parts of each frequency for the right and left hemispheres EEG channels respectively.
6. The similarity between the topography of each alpha frequency activity of the recorded data with the topography of the 40 Hz amplitude modulated signal is an index of the extent to which the alpha oscillation from non-auditory cortex sources are active. Due to the fact that these topographies are two non-zero vectors, we use the inner product which divulges of the one vector is pointing in the direction of the other one. The inner product, as a measure of similarity between these two non-zero vectors for two pairs, is calculated and the maximum value of each pair is selected as the similarity of right and the left hemispheres respectively for the specific frequency F (equations (4.5) and (4.6)).

$$SI_{RF} = max\left(\langle U_{RL} \bullet U_{1RF}\rangle, \langle U_{RL} \bullet U_{2RF}\rangle\right) \quad (4.5)$$

$$SI_{LF} = max\left(\langle U_{LL} \bullet U_{1LF}\rangle, \langle U_{LL} \bullet U_{2LF}\rangle\right) \quad (4.6)$$

U_{RL} and U_{LL} are the topographies in 40 Hz for the right and left hemispheres. U_{1RF} and U_{2RF} are the first and second columns of u in equation (4.1) for frequency F of the right hemisphere. U_{1LF} and U_{2LF} are the first and second columns of u in equation (4.1) for frequency F of the left hemisphere. SI_{RF} and SI_{LF} are the similarity indices for the right and left hemispheres for frequency F respectively. The dot $\langle \bullet \rangle$ is the inner product.

7. The optimal EEG channels were also divided into the left and right hemispheres. EEG midline channels were assigned to both the left and right hemispheres. The power for the EEG channels for the left and right hemisphere was calculated for each frequency in alpha band (8-13) with 1 Hz resolution. Then, it was multiplied by its own similarity index (equations (4.7)

and (4.8)).

$$FP_{LF} = Power_{LF} \times SI_{LF} \quad (4.7)$$

$$FP_{RF} = Power_{RF} \times SI_{RF} \quad (4.8)$$

$Power_{RF}$ and $Power_{LF}$ are the power in frequency F for the right and left optimal channels. FP_{RF} and FP_{LF} are the filtered power in frequency F for the right and left optimal channels.

8. Since the frequency resolution is 1 Hz, we had six filtered powers for left hemisphere and six filtered powers for the right hemisphere. The total filtered power for both left and right hemispheres could therefore be calculated (equation(4.9)) .

$$TFP = \sum_{F=8}^{F=13} FP_{LF} + \sum_{F=8}^{F=13} FP_{RF} \quad (4.9)$$

TFP is the Total Filtered Power for one segment of data (1 sec) for alpha band (8-13Hz) with 1 Hz resolution. F is a frequency in alpha band from 8 to 13 Hz.

4.2 Results

Using simulated data to recognize alpha sources originating from non-auditory and auditory brain regions, we will first demonstrate the efficiency and validity of the similarity method. We will then proceed to demonstrate the method of identification of auditory cortex sources in the alpha band of spontaneous cortical rhythms in 3 tinnitus patients during the neurofeedback paradigm.

4.2.1 Simulated Data

We simulated sources in the alpha rhythm using the FiledTrip Toolbox [138] in different locations inside a regular grid. The aim was to ascertain how the similarity method recognizes that the sources do not originate from primary auditory cortices. This was determined by the extent to which the pattern of each source resembles the pattern of 40

Hz sources as representative of ASSR. We used an analytical concentric sphere model with 3 spheres [139] for forward calculation. There are two sequences of simulations:

1. Having defined a head model, the grid points of a regular grid inside the 3 spheres with 1 cm resolution are selected. Based on this resolution there are some dipoles inside and outside the spheres. The position of those dipoles which are located inside the spheres are selected for reconstruction of electrode-level EEG data. There are two main pair sources, the first pair, 40 Hz frequency sources on both primary auditory cortices and the other 10 Hz frequency sources in left and right primary auditory cortices representing auditory alpha. The momentum of the left 10 and 40 Hz sources is the same and also the momentum of the right 10 and 40 Hz sources is the same. The other remaining 10 Hz sinusoidal sources are located in non-primary auditory regions to simulate spontaneous alpha oscillatory brain activity outside primary auditory cortices. Firstly, the forward model is calculated for both left and right 40 Hz sources and then in the same location, the forward model for two 10 Hz sources is computed. Then, the forward models are computed for all remaining sources with different locations compared to the two main pair sources. As each source can have a different phase and momentum, the number of forward models for each source with a specific location is unlimited. Although it is impossible to compute all forward models for all combinations of phase differences between primary auditory and non-primary auditory sources, we certainly cover a great many possible cases to check the efficiency of this method in distinguishing between primary auditory and non-primary auditory alpha sources. After the calculation of all forward models for all sources, the similarity method is applied to each forward model to investigate to what extent the brain pattern from each source activity resembles the pattern from ASSR-simulated sources. For the two 10 Hz sources, their similarity indices are displayed in the left and right source positions (Figure 4-4). For all other sources, the average of the left and right hemispheres' similarity indices is displayed in source locations (Figure 4.4). Five sets of data are stimulated. In the first data set (A), all non-auditory

sources have the same phase as the right 40 Hz source and all their momentums are the same as the right 40 Hz source. In the other four remaining datasets (B, C, D, and E) all non-auditory sources have 45, 90, 135, and 180-degree phase difference with the sources located in the primary auditory cortex but their momentums are the same as the right 40 Hz sources. The result of the five simulated data sets is shown (Figure 4-4 A-E).

2. The ASSR forward model is based on both the left and right 40 Hz sources, which highly correlate, [136] leading to the phase difference between them being very small. Therefore, there is only one topography for the two 40 Hz sources. However, the scenario for the left and right 10 Hz sources is different, because there might be a phase difference between the two, which in turn leads to different topographies. Hence, one simulation of data from only the two 40 Hz sources with zero phase difference is calculated, but the phase difference between two 10 Hz begins with 0 degrees and gradually increases in 10-degree steps until the difference reaches 180 degrees. The forward model is first calculated for the two 40 Hz sources, and then in each step, the forward model is calculated for the two 10 Hz sources, followed by the similarity method which compares the patterns of two 40 Hz and two 10 Hz. The similarity index for each source is presented with a specific color in the location of the source (Figure 4-5).

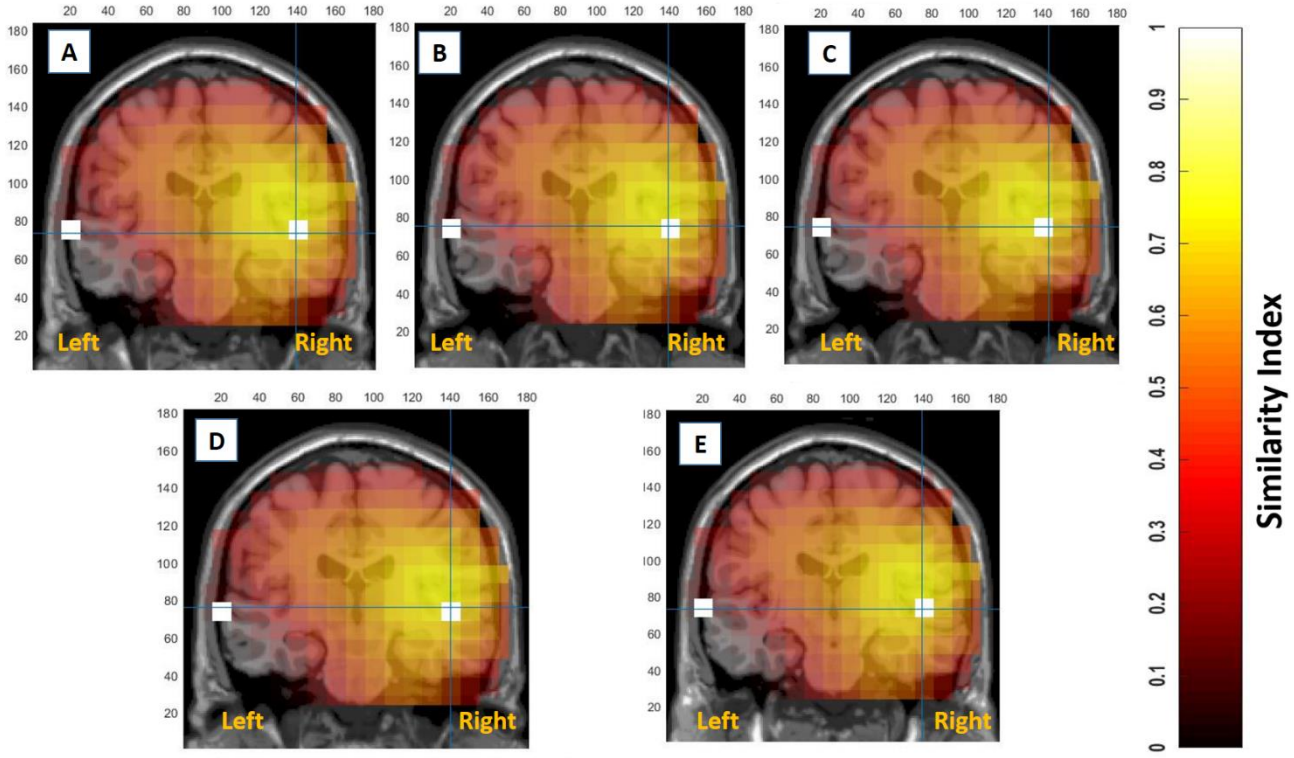

Figure 4-4: Result of the similarity index calculations for the first simulated data sets A, B, C, D, and E. In all data sets, the similarity indices have the highest value in the auditory cortex. The result shows that the closer the sources are to the auditory cortex, the higher are the values for similarity indices. Since the momentum of all non-auditory cortices in this data sets is the same as in the right auditory cortex, the high simliartiy values are mostly located on regions adjacent to the right auditory cortex. In the first dataset (A), the phase difference bewteen auditory and non auditory sources are zero. In the second dataset (B), the phase difference is 45 degrees, in the third dataset (C) the phase difference is 90 degrees, in the fourth dataset (D), the phase difference is 135 degrees, and in the fifth dataset (E), the phase difference is 180 degrees.

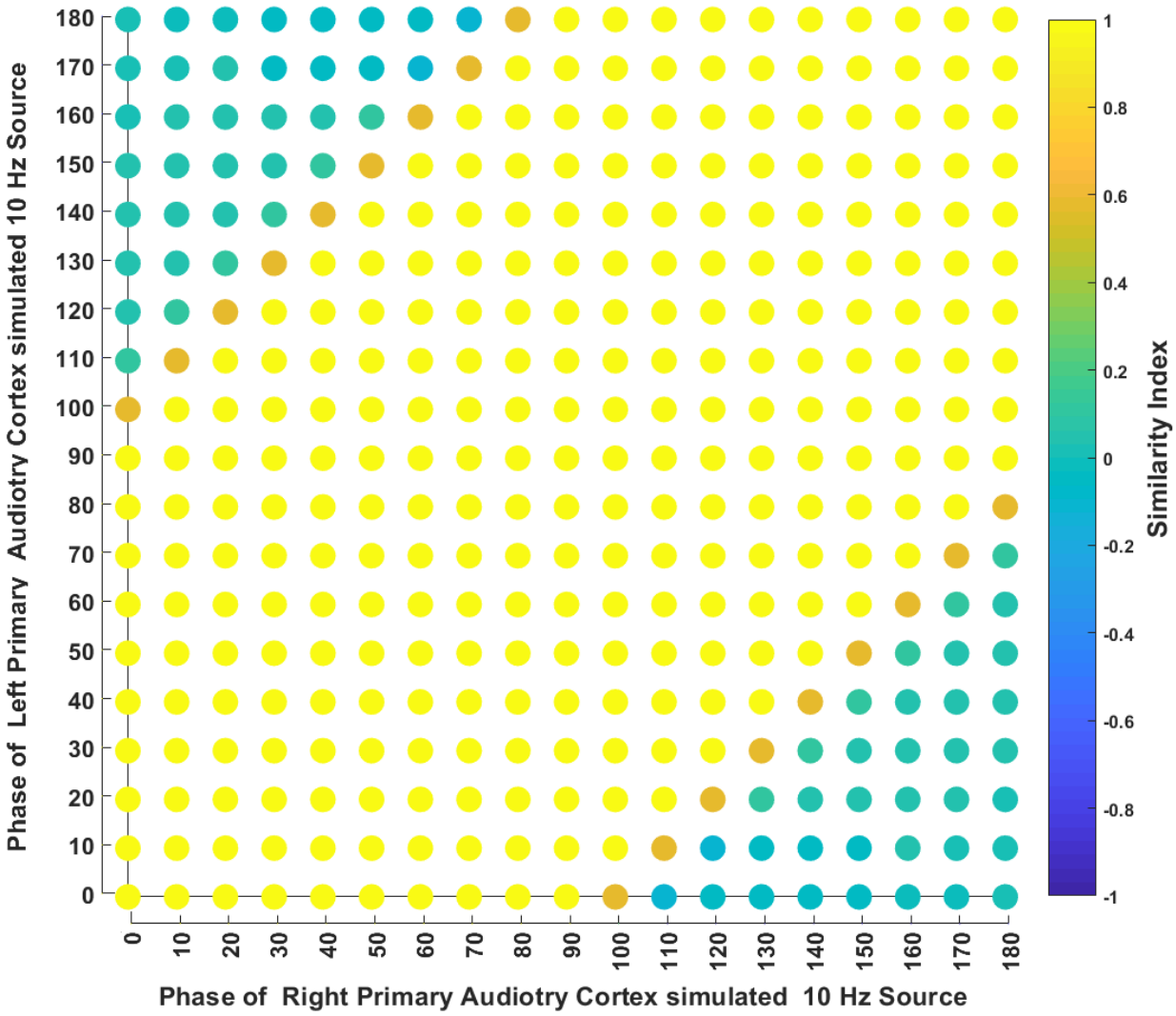

Figure 4-5: Result of the similarity method regarding the phase difference between two auditory 10 Hz sources. The resolution is +10 degrees. The x-axis is the phase of the right primary auditory cortex simulated 10 Hz and the y-axis is the same for the left primary auditory cortex. The color bar on the right side indicates the mapping of similarity indices in the color map. The similarity indices are negative in the top left and the right bottom of the figure.

4.2.2 Localizer Runs

The result of the localizer runs specifies those channels that reflect auditory cortex activity. The occipital and visual patterns and the difference between these two patterns for these three patients are depicted (Figure 4-6). Each pattern consists of an array which

assigns a negative or positive value to each channel. Only the absolute value of each element in each array shows how much that channel reflects activity in that pattern. Therefore, we used the absolute value of each pattern. Auditory pattern reflects its own activity in central and frontal regions, visual pattern reflects its own activity in the occipital and frontal regions. The difference of these two patterns is the result of the subtraction of these two patterns, which cancels out those regions in which both patterns show high activity (the frontal region). The optimal channels selected in these patients are consistent with those channels selected in other EEG neurofeedback studies for tinnitus patients [129,140]. A further consistency with other auditory studies, in particular with steady state response sources analyses is that selected channels are in those regions of the brain towards which auditory cortex dipoles are oriented [141,142]. Table 4-2 shows the optimal EEG channels for the three patients.

4.2.3 Similarity method with physiological data

We postulated that the similarity method reflects the auditory cortex activity without using any standard source localization methods. The total filtered power (TFP) for one segment of data (1 sec) for alpha band (8-13Hz) with 1 Hz resolution was calculated using equations of the subsection (4.1.4.2). All TFP values for all three visits were sorted from minimum to maximum and divided into 30 bins where each bin corresponds to a specific range of TFP. Then all the segment data (1 sec) which corresponds to each bin were extracted from the regulation phase data. Source localization for all the MNI (Montreal Neurological Institute) atlas cortical regions were then performed for all segments in each bin and for each frequency in alpha band. The regions of interests were right temporal, left temporal, right auditory cortex, left auditory cortex and the regions of no-interest were right frontal lobe, left frontal lobe, right occipital lobe, left occipital lobe, right paracentral-central, left paracentral-central, parietal, right parietal and left parietal. We calculated the volume conduction model of the template MRI of Fieldtrip data set on the basis of boundary element method (BEM) [143]. We then used one of the beamforming source localization methods, dynamic imaging of coherent sources (DICS) [127] to calculate the power of alpha band (8-13Hz) with 1 Hz resolution at source level in the MNI cortical brain regions. To compare alpha power of one bin to the other in one cortical region, the

alpha power in that region is divided to the sum of alpha power in all cortical regions. This allows the comparison of the relative alpha power across the bins. The results over the different frequencies in the alpha band were averaged and are displayed (Figure 4-7). The distribution of the averaged SI_{RF} and SI_{LF} across all frequency F with 1 Hz resolution in the alpha band for each patient and across these three patients is shown (Figure 4-8).

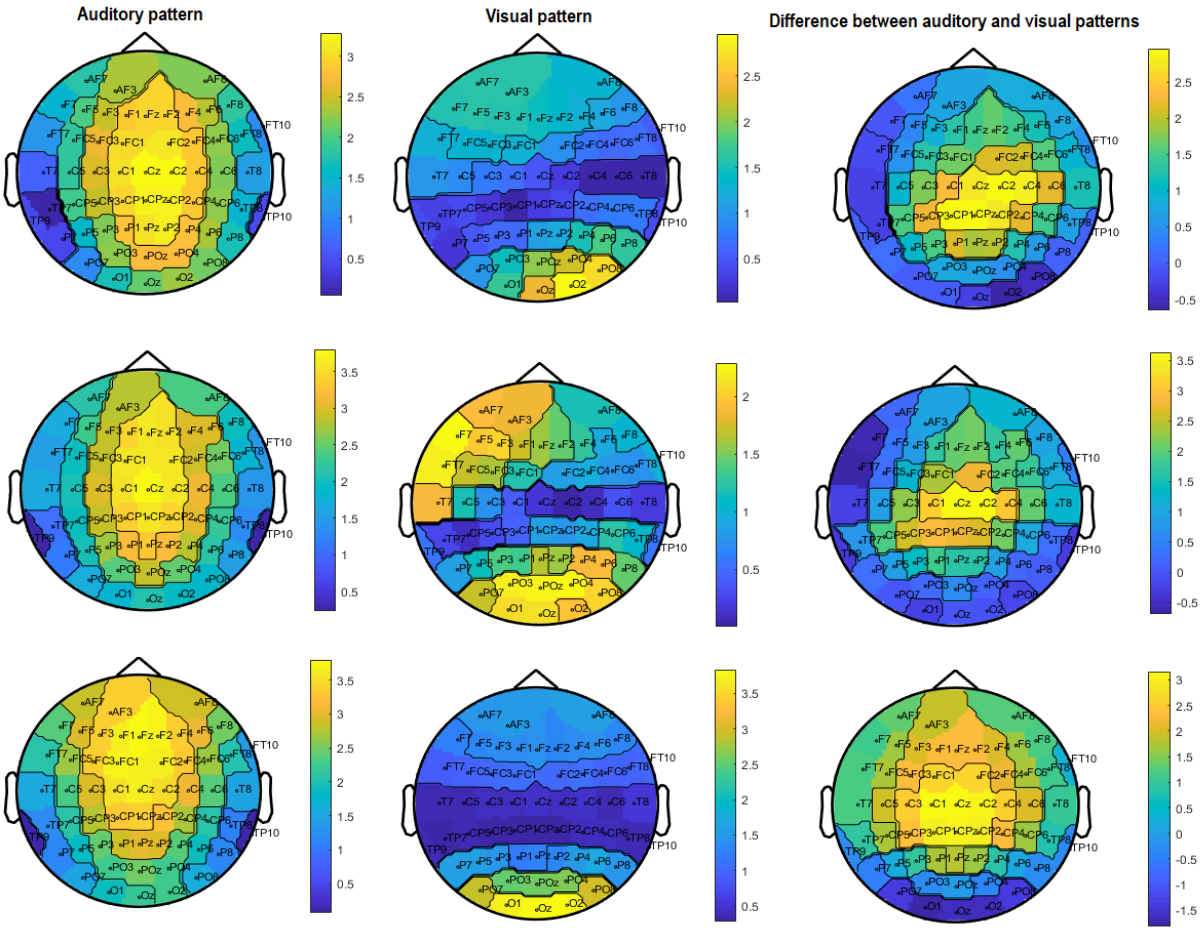

Figure 4-6: Topography decomposition for the most relevant channels reflecting auditory and occipital activity. Each row corresponds to one patient. The first and second columns show the auditory and occipital patterns for three patients. Each pattern consists of an array which assigns a negative or positive value to each channel. Only the absolute value of each element in each array shows how much that channel is reflecting activity in that pattern. Therefore, we used the absolute value of each pattern. The difference of these two patterns which is the result of the subtraction of these two patterns (last column). In the third column auditory channels are those with high positive values in the fronto-central and centro-parietal regions, while the occipital channels are those with high negative values in the occipital region. The description of how the specific channels can be selected is included in the subsection (4.1.4.1).

Table 4-2: Channels mainly reflecting the activity from auditory cortex

Patient	
1	FC2-FC4--C1-Cz-C2-C4-C6-CP1-CPz-CP2
2	FC1-FC2-C1-Cz-C2-C4-CP1-CPz-CP2
3	FC1-FC2-FC4-C3-C1-Cz-C2-C4-CP1-CPz-CP2

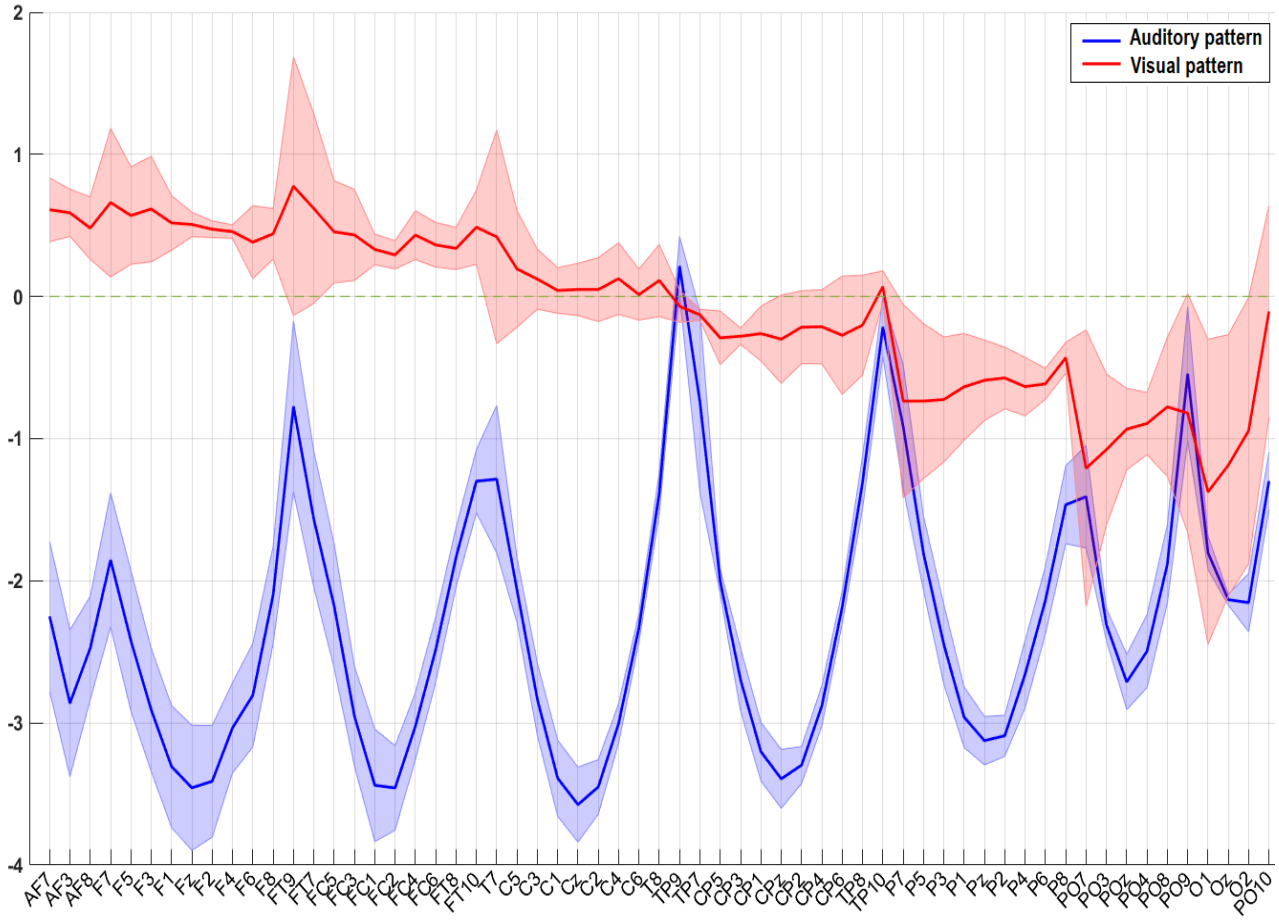

Figure 4-7: Topography decomposition for reflecting auditory and visual patterns across three patients. The red line depicts the occipital activity pattern (shadow is the standard deviation) and the blue line the auditory activity across three patients (shadow represents the standard deviation). The absolute values of y-axis reflect how much the component contains auditory or occipital activity (no unit).

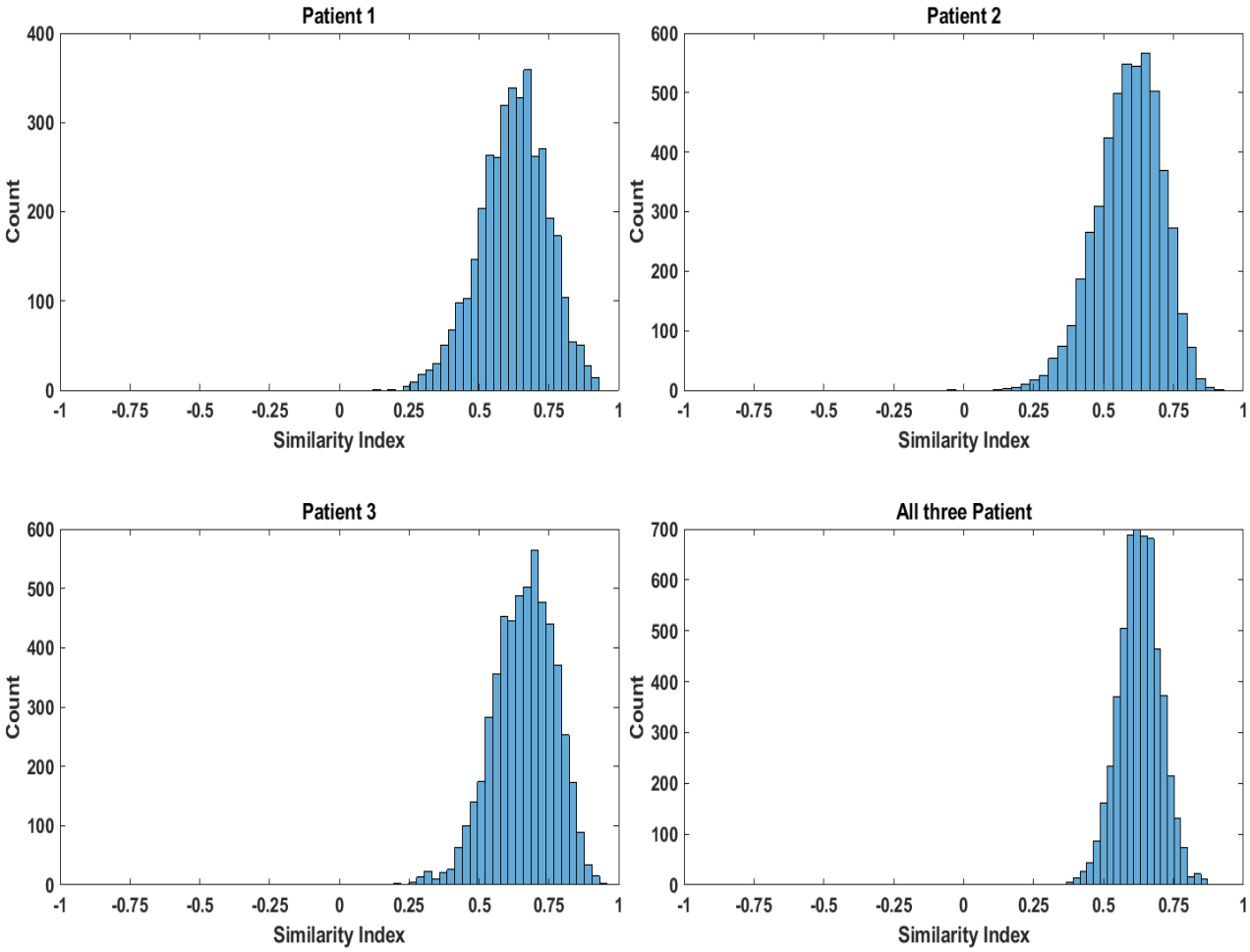

Figure 4-8: Histograms of averaged total similarity index (left and right hemispheres) for all frequencies in the alpha band (8-13 Hz) with 1 Hz resolution for each patient and the right lower plot is the histogram of total similarity index across three patients. The x-axis represents the similarity index and the y-axis is the count which corresponds to each bin.

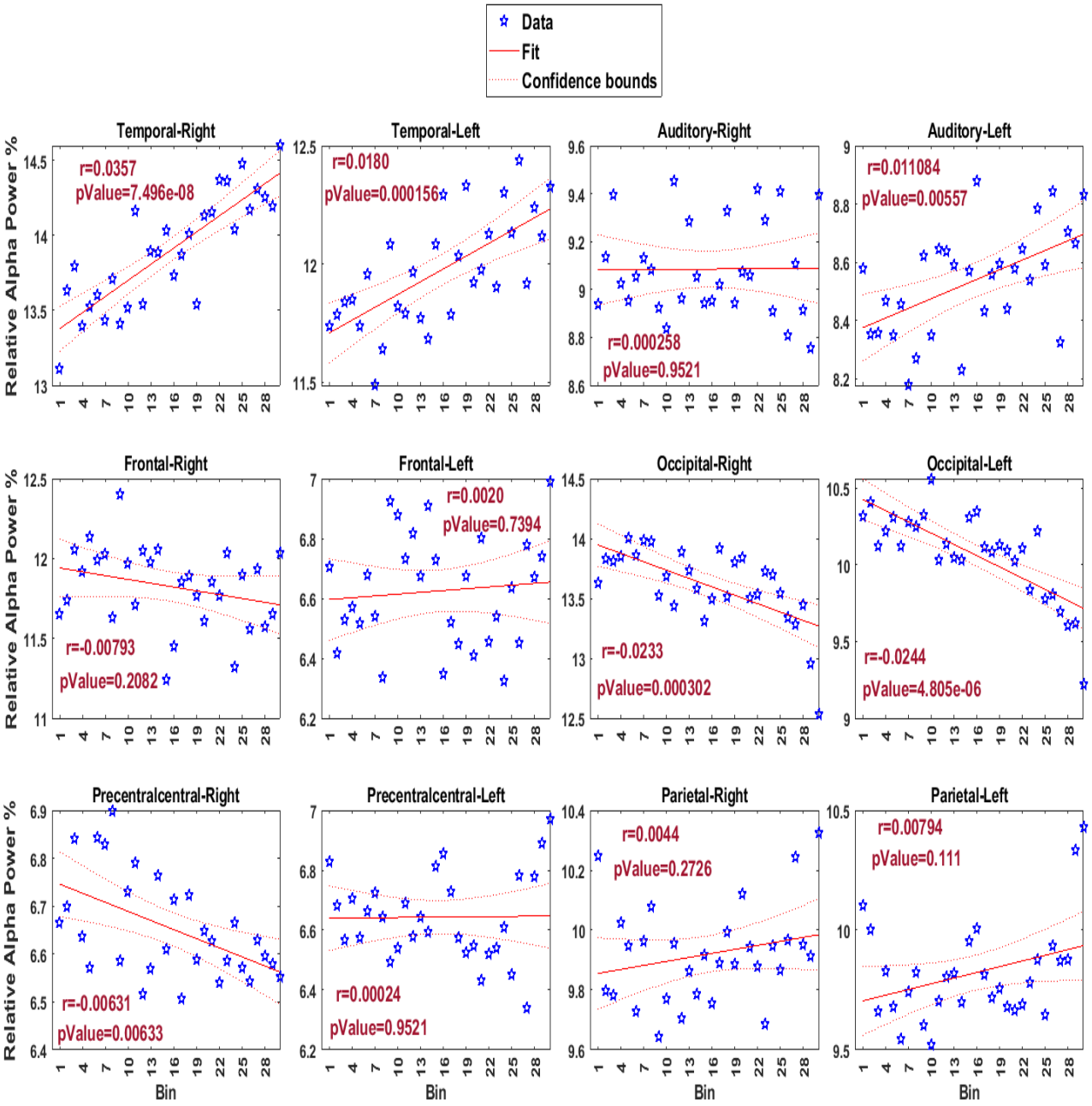

Figure 4-9: Relative alpha power of each cortical brain region (based on the MNI atlas coordinates). Relative alpha power is the alpha power of each cortical region in each bin (a TFP range) divided by the sum of alpha power in all cortical regions in the same bin (details in subsection (Similarity method with physiological data). The x-axis displays up to 30 bins. Each bin corresponds to a specific TFP range (corresponding to 158 segments, 1 second each) of the alpha-regulation phase data. The y-axis shows the percentage of relative alpha power of a specific brain region. The corrected p-values using the Benjamini & Hochberg method [144] for each region and the slope of the fitted line are shown in each subpanel. The regions of interests are right temporal, left temporal, right auditory and left auditory.

4.3 Discussion

Real-time source localization of EEG data is a challenging procedure. The low signal to noise ratio, the time-consuming procedures of the inverse solutions and the limited time points of the data make a real-time source localization difficult to apply in real-time EEG neurofeedback. We therefore implemented a novel similarity method which functions as a spatial filter to prevent alpha feedback from non-auditory cortices.

The effectiveness of this method has been established with both simulated and physiological EEG data. Figure 4-4 (A-F) demonstrates that the similarity indexes are positive in the auditory cortices and the adjacent regions only. Although the adjacent regions to auditory cortex have high similarity indices, presenting tactile stimulation during actual EEG neurofeedback supposedly suppresses the alpha activity in these regions. Furthermore, the phase difference between non-auditory sources and both ASSR sources ranges from 0 to 180 degrees in 45-degree steps, showing that the similarity method is also robust to phase differences. The other factor which should be considered is the phase difference between two 10 Hz sources in the auditory cortices. Since the phase difference between two 40 Hz sources as highly correlated sources is very small, which leads to having only one ASSR topography. Therefore, only one ASSR topography was compared with different alpha auditory topographies in the second phase of simulation. Figure 4-5 shows the effect of the phase differences among the two main 10 Hz sources. It can be seen that the similarity method tolerates phase differences between the two 10 Hz sources approximately up to 100-degree.

Since the similarity method recognizes the auditory activity at the sensor level, the channels from which the alpha feedback is passed on to the patient must be specified. Table 4-2 shows the optimal channels for these three patients which are in the centro-parietal, central, and fronto-central region. Figure 4-6 demonstrates auditory and visual patterns. The auditory pattern manifests its activity neighboring the midline electrode from parietal to the frontal areas, while the visual pattern displays its activity in occipital and frontal regions. The difference between these patterns shows high positive values in

the central area and high negative values at the occipital areas (the last column of Figure 4-6). This in turn determines the optimal and occipital channels respectively.

Each training session lasted 2 hours including preparation and required focused attention. We reduced this amount of time to a maximum of 1 hour for the second phase of the study by reducing the number of EEG channels, which might influence the accuracy of the similarity method. Hence, following the selection of optimal channels, a number of channels reflecting occipital activity were also selected to compare -to some extent- the whole pattern of brain activity during real-time neurofeedback with the ASSR pattern acquired during the first visit (localizer run 1).

It should be emphasized that with this small number of participants we cannot statistically test the variability across these patients. But as these tinnitus patients have been recruited based on the specific inclusion criteria, like a Tinnitus Handicap Inventory (THI) above 48, the variability across them, might be very small. From figures 6, 7, and 8 could be seen that the manifestation of auditory and visual patterns, optimal channel location and range of similarity indexes are to some extent the same for these patients. In figure 8, the bins with similarity index value more than 0.8 have very low counts, meaning that the non-auditory alpha sources are still active and contribute to global alpha power at the sensor level. The ideal situation would be that all non-auditory alpha sources are inactive. This would lead to a maximum similarity index as shown in the simulated data section. Relative alpha power of each cortical brain region (Figure 4-9) shows that, although the similarity method effectively reduces the contribution of alpha power of some of the non-auditory cortices to the alpha power calculated on the sensor level, the increase in TFPs is not accompanied by an increase in alpha power in the right auditory cortex.

The increase in TPFs leads to less alpha contribution of non-auditory areas at the electrode level. However, increasing alpha source power in the auditory regions is a matter of training. The reason behind alpha power having not increased in all regions of interest might be low similarity indices in these three visits. More sessions should solve that problem as in all skill-learning procedures characterizing neurofeedback learning [145]. With increasing session number, the specificity and anatomical focus of the change becomes more specific and circumscribed.

Chapter 5

5 EEG alpha neurofeedback with emphasis on auditory cortex sources in Chronic Tinnitus

In this chapter a preliminary study will be described in which, we used the Similarity method, which was comprehensively explained in the last chapter. The approach used in the present study differs from previous EEG neurofeedback studies not only due to its focus on the auditory cortex alpha band power but also to its innovative setup enabling the suppression of visual and somatosensory alpha activity through visual stimulation and air flow as tactile face-stimulator. Furthermore, the experimental procedure is novel due to the inclusion of pink noise, also known as 1/f noise, as acoustic tinnitus masker. The aim of this current study is to determine the effectiveness of this treatment approach and to discuss benefits and limitations.

5.1 Method

5.1.1 Participant recruitment and demographics

17 chronic tinnitus participants were recruited by advertisement and clinical referral. In order to be eligible for study inclusion, patients had to be diagnosed with chronic subjective tinnitus (> 0.5 years). Five participants were excluded from this study. The reason for exclusion two of them was the EEG data of localizer visit contaminated with muscle activity and head movement, therefore we could not be able to select optimal channel properly. Two patients could not finish the study. The last one had a leg operation after three weeks of neurofeedback training and then, he had to stay at home for at least one month. Therefore, we decided to exclude him from the study. The twelve participants

with the age (56.7±13.2) and tinnitus duration in year(9.5±7.2), were German native speaker, suffer from no other psychiatric or neurological disorder, and have no acute suicidal tendency. Furthermore, patients with drug or alcohol addiction, cochlear implants, and current prescriptions for tranquilizers, neuroleptics, or antiepileptic were not considered. Table 5-1 summarize the demography of these 12 patients.

Table 5-1: Demography of the chronic tinnitus participants recruited for this study

Number	Sex	Age	One or both side	Tinnitus duration (year)
1	m	54	both	4
2	m	57	both	20
3	m	43	left	1.5
4	m	54	both	2
5	m	52	both	2.5
6	m	67	right	12
7	m	75	left	10
8	m	65	both	11
10	m	54	both	16
11	m	37	both	18
12	m	28	both	1
13	m	72	both	2

5.1.2 Material

On the localizer and resting state data collection visits the actiCAP with its 64 active electrodes (based on high-quality Ag/AgCl electrodes) was placed with a standard EEG-cap using the Brain Products recording system (BrainAmp) (Figure 5-1C). On the neurofeedback visits the actiCAP was used but the number of active electrodes was different from patient to patient (Table 5-3). The location of the reference electrodes were the same as localizer visit. Also, for the eye movements monitoring during neurofeedback session, the same numbers and positions of the electrodes were as the localizer visit.

5.1.3 Experimental design

This prospective clinical trial consisted of 17 visits in total (Figure 5-1 A), 2 visits, first and the last visits for audiometric screening and the relevant questionnaires. Visit number 2 was a localizer visit by which some specific EEG channels were selected for each patient individually and 14 visits for neurofeedback training. In the first appointment, 1-2 weeks before the start of the neurofeedback training visits, the patient was in an interview informed about the purpose and procedure of the study and signed his/her informed consent in the presence of a qualified medical professional. In the same visit, participants further underwent the audiometric screening in which audiometric measurements were realized. At the same visit patient was asked to complete five questionnaires covering demographics and tinnitus-related symptoms, as well as several other psychological and health-related questions. The five questionnaires were Tinnitus Handicap Inventory (THI)[146], State-Trait Anxiety Inventory (STAI-T)[147], Pittsburgh Sleep Quality Index (PSQI)[148], World Health Organization Quality of Life (WHOQI)[149] and Beck Depression Inventory (BDI)[150]. If the patient has passed the inclusion and exclusion criteria (Table 5-2:), he/she could participate in one localizer visit and total of 14 neurofeedback training sessions, one or two times per week, and one week after the completion of the training period as the last visit, a post-measurement was performed consisting of the repeated measurement of the questionnaires and doing audiometric test. The schema depicting the experimental procedure, acquisition, and analysis EEG neurofeedback visits is shown in Figure 5-1.

5.1.3.1 Eye movements runs

In the second visit eye movements runs were performed. The aim of eye movement calibration run was to specify a threshold for horizontal and vertical eye movements for online and offline rejection of EEG data. Two reference electrodes were attached on both mastoids. In order to monitor vertical eye-movements, two electrodes were placed infra-orbital and supra-orbital to the right eye. One EEG electrode was placed 1cm lateral to the left outer canthus. The electrical activity of this electrode was then subtracted from the average of the other two infra-orbital and supra-orbital electrodes resulting in horizontal eye-movements activity. The eye movement calibration run consisted of two

phases; a horizontal eye movement phase in which the patient had to look at the left and right part of the screen 10 times (5 times left and 5 times right), and a vertical eye movement phase in which the patient had to look at the upper and lower part of the screen 10 times (5 times up and 5 times down).

5.1.3.2 Localizer and resting state

The Localizer visit as a second visit had to cover three purposes: firstly, to find a brain pattern when primarily auditory cortex is stimulated via an auditory stimulus, secondly to select the most relevant channels which were selected as the optimal channels for neurofeedback and thirdly to define a threshold of horizontal and vertical eye movements to reject EEG signals in offline and online preprocessing procedures. The second run of localizer visit in which patients' eye is open was also considered as the first resting state. We recorded resting state also in visits nine and sixteen in the same way as runs 2 in localizer visit.

5.1.3.3 Neurofeedback sessions

The neurofeedback system and method is the same as it has been described in the last chapter. But here it will be also explained succinctly.

In each EEG neurofeedback training visit each patient performed 4 runs which each run lasted 10 minutes including 20 trails. Each trial has three phases, rest, pink noise and regulation phases. During a resting period, 5 second, the patient was instructed to count downwards from 100 with -5 steps. In the second phase, pink noise (8-10 second) is behaving as acoustic tinnitus masker. During this interval the patient is allowed to blink to avoid any blinking in other interval. During a regulation phase 12 second, patient tried to increase in length a thermometer in the middle of the screen in horizontal direction which reflects the alpha increase compared to the rest phase (Figure 5-1 B). The segment of data for real-time analysis was 1 sec and the moving window was 500 milliseconds. The feedback was updated every 500 milliseconds. The given feedback is the product of

alpha power in selected channels the similarity index which shows how much the alpha from primary auditory cortex is contributing to the feedback. Appling the similarity index on the alpha calculated from optimal channels gives a filtered alpha power which reflects mostly alpha activity originating from auditory brain area.

There were two reasons for the pink noise phase. Firstly, it gave the patient time to blink or move the head if necessary. Secondly, in the pink noise phase the patient was presented with the specific auditory stimulus (pink noise) which intends to gives the patient a relief of tinnitus through masking the tinnitus[134]. It could also suppress the alpha oscillation in the auditory cortex during its presentation, which should lead to a rebound of alpha oscillation in the regulation phase. This rebound can exceed the pre-stimulus baseline level of alpha oscillation[135] facilitating alpha upregulation for the patient in the regulation phase. If the patient blinks and the threshold criteria was met in other intervals the feedback is not updated and that segment of data (500 millisecond) is not undergoing online analysis. After each trial the patient is presented with a score which shows the neuro-feedback result for that specific trial. Additionally, during the rest and regulation phase, we presented a tactile stimulation which consisted of an air flow with constant velocity that stimulated the mental protuberance which should block sensory-motor alpha-rhythm (SMR). The stimulation of the protuberance should suppress the alpha activity in somatosensory regions adjacent to the auditory cortex. We hypothesize that suppression of somatosensory alpha through tactile stimulation and suppression of occipital visual alpha from the visual stimulation of the visual feedback signal at the screen facilitates auditory alpha self-regulation.

One of the major points in learning procedures is increasing the difficulty of learning process over time. For this purpose, the horizontal red bar (Figure 5-1 B) in the regulation phase, in the first visit of neurofeedback was filled with one Signal Percentage. Signal percentage was calculated with the equation (5.1) :

$$\text{Signal Percentage} = \frac{(\text{Filtered alpha in regulation} - \text{Filtered alpha in rest})}{\text{Filtered alpha in rest}} \quad (5.1)$$

After each run if the subject performance went beyond the initialized value (1* Signal percentage), we put a new signal percentage in the software for neurofeedback leading to the more difficult task during the next regulation phase.

To facilitate transfer of the self-regulation skill to everyday life, in our neurofeedback protocols we used transfer runs. Transfer runs are where no feedback is provided during the given period, this process refers to a response generalization. Therefore, after visit number 9, two runs of each visits were randomly selected as transfer runs.

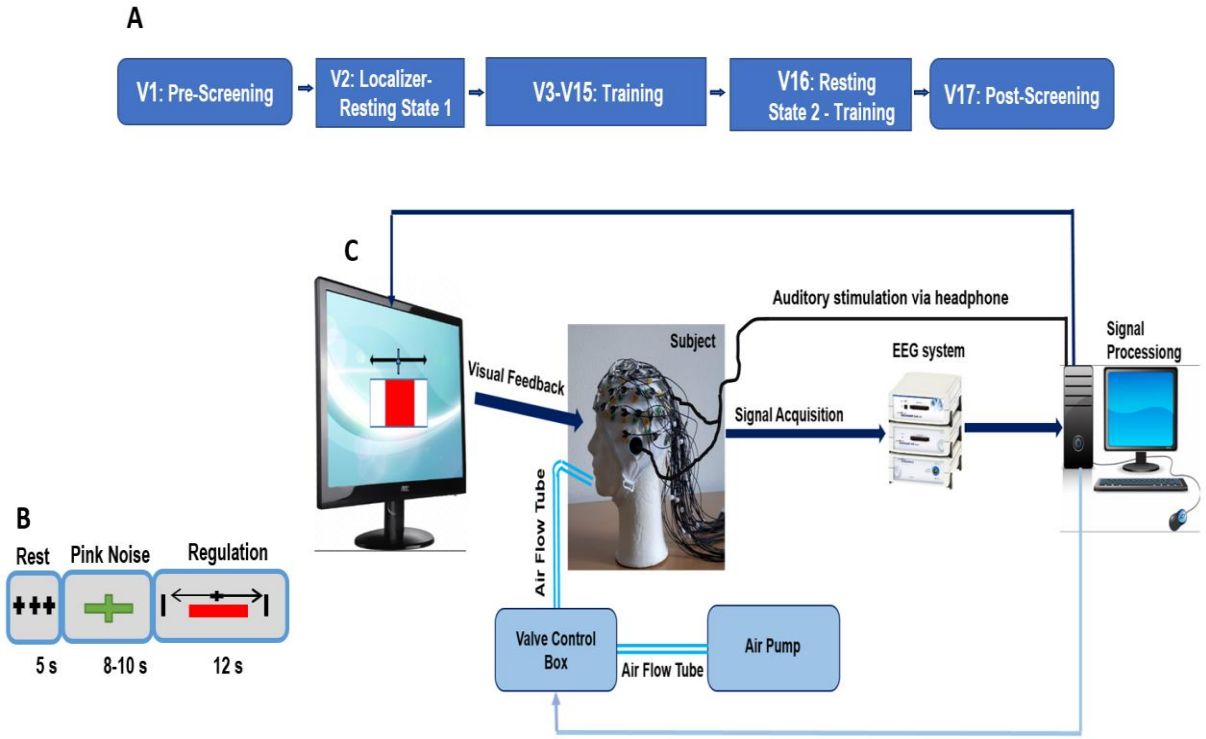

Figure 5-1: **A**) Schedule of assessments and **B**) Experimental protocol. The patient receives 20 trials per run. Each run starts with 5 sec rest (black cross on a gray background). It is followed by 8-10 sec of pink noise phase which is cued by a green fixation cross in the center. During this phase, pink noise is presented to the patient and he/she is allowed to blink or to move his/her head. Then, there is a 12 sec regulation phase, cued by two black horizontal arrows. During this condition, a red horizontal thermometer will be extended/shortened from the center of the screen based on the amount of the alpha activity coming from the auditory cortex. In addition, during rest and regulation phases, a tactile stimulation consisting of airflow with constant velocity stimulates the mental protuberance. **C**) Experimental situation: Neurofeedback setup including EEG, an air-pump for the tactile stimulation of the mental protuberance, auditory stimulation via earphones and hardware for online EEG signal processing.

Table 5-2:: Inclusion and exclusion criteria of Tinnitus patients

Inclusion criteria	**Exclusion criteria**
1. Age between 18 to 80 years 2. Chronic, persistent, non-pulsatile tinnitus since a minimum of 6 months 3. Normal functional hearing 4. Normal inner ear structure findings using an ear microscope, normal tympanic membrane mobility 5. Participant willing, able and available to participate to the entire research, including completion of questionnaires and traveling to research sites for the duration of the trial	1. Contraindication to MRI (e.g. non compatible cochlear implant, pacemaker, deep brain stimulation) 2. Conductive hearing loss exceeding 20 dB at two or more frequencies 3. Known diagnoses causing tinnitus or hearing loss: • Known systemic disease (vestibular schwannoma, endolymphatic hydrops) • Lesion in central nervous system, including history of severe cranio-cerebral trauma • Acute ear canal or middle ear inflammation or effusion 4. Significant neurologic disease, psychiatric disease, substance abuse or acute allergic disease 5. Ongoing medication that is known to treat, influence, or cause tinnitus (e.g. high-dose aspirin, quinidine, aminoglycosides) 6. Ongoing or recent (completed since less than 4 weeks) tinnitus therapy (e.g. tinnitus maskers, acupuncture) 6. Participation in competitive or pharmacological study

5.2 Analysis and statistic

5.2.1 Offline analysis at sensor and source level

The first step in analyzing EEG data is to control the EEG signal quality in the sense of not contaminated with physiological/biological or nonphysiologically artifacts, resulting from electrical phenomena or devices in the recording environment. Physiological artifacts may include cardiac, pulse, respiratory, sweat, glossokinetic, eye movement (blink, lateral rectus spikes from lateral eye movement), and muscle and movement artifacts. Non-physiological / Technical artifacts may include Electrode pop, Cable movement, Incorrect reference placement, AC electrical and electromagnetic interferences and Body movements[151]. Firstly, the data for each patient was visually checked whether to contain any kind of the mentioned artifacts. Those trials or channels

which contain muscle, movement artifacts or technical artifacts are removed from the data. From the localizer visit a threshold for vertical and horizontal eye movements were calculated. Secondly, for each segment, 1 second (500 samples), the vertical or horizontal EOG signal of regulation, rest phase and localizer were calculated. If the EOG signal contained more than 200 samples which their own amplitude was larger than 80 percent of the maximum vertical or horizontal eye movement calibration run, the segment was excluded. Thirdly, a method for automatic EOG artifact correction using adaptive filtering was applied, if the EOG signal of one second contains 10-20% of 80 percent of the absolute maximum of the vertical and horizontal eye movements[152].

5.2.2 Localizer visit analysis

The Localizer visit aimed at three objectives,1) to obtain an Auditory Pattern (AP) of brain activity which represents the presence of auditory cortex activity sources and is used as a spatial filter which prevents patients receiving non-auditory alpha feedback during the regulation phase. 2)To find optimal channels from which the patient's feedback is given. 3) to determine a threshold for vertical and horizontal eye movements for rejection or correction of EEG data in real time and offline analysis.

As the preparation of a 64 EEG-electrodes 'cap lasted roughly one hour, we decided to use a smaller number of channels for training visit which in turn decreases the precision of the similarity method. Therefore, some channels are selected which manifest visual activity for improved estimation of brain topography during training visits. But from only optimal channels a patient is given feedback. The steps in obtaining AP and optimal and visual activity channels are explained in detail in the last chapter.

5.2.3 Sensor level analysis of the neurofeedback visits

The segment of data for analysis was 1 sec and the moving window was 500 milliseconds. The similarity method[32] was applied in the alpha band (8-13Hz) of the feedback and resting phases. Individual normalization was carried out such that the result of similarity method of 1 second feedback phase is subtracted and divided by the average of the result

of the similarity method on the resting phase for that trail. Then the result was firstly averaged over trials and then over run and finally over day for each patient individually. Then using Wilcoxon signed rank test the result of neurofeedback for each patient, for 14 visits is tested against a normal distribution with mean equal to zero and unknown variance. Based on this analysis we defined three subject types: **Type A1**) one who could keep the level of alpha feedback above baseline during the training visits and its learning curve has positive, significant slope, **Type A2**) one who could keep only the level of alpha feedback above baseline during the training visits but its learning curve does not has positive, significant slope, **Type B**) one who could not keep the level of alpha feedback above the baseline during the training visits. We named the first type A1 actual learner patient and type A2 potential learner patient and type B non- learner.

The other analysis which was performed on all training visits was to calculate the similarity index based on formulas number 5 and 6 from the chapter 5 and then averaged over left and right hemispheres for each segment of the regulation phase (1second) for all training visits and all 12 patients. The similarity index is an indirect indication of how much non-auditory sources during regulation phase are active contributing to alpha activity recorded in the optimal channel. The maximum of similarity index is [**1]** and minimum is [**-1]**. Closer to 1 corresponds to less non-auditory active source.

The other analysis which was performed only in the learner group, was to compare the transfer runs with training runs in order to examine whether the learning skills are not associated with the neurofeedback apparatus or the situation in which the neurofeedback is performed. In order to perform this analysis, relative alpha power calculated as explained above of transfer runs then the way repeated measure Anova (Analysis of Variance)was applied .

As during the pink noise phase, the patient was exposed tothe specific auditory stimulus pink noise in order to reduce the discomfort caused by tinnitus. Furthermore, pink noise could suppress auditory alpha activity during this period which in turn leads to alpha rebound in the auditory area in the regulation phase which might facilitate up-regulation. As in training session number 8 and 15 we used 64 EEG electrodes to investigate the effect of the pink noise on the source level, we used Standardized low-resolution brain

electromagnetic tomography (sLORETA)[128] to explore whether alpha activity in auditory cortex has been suppressed compared to the regulation phase. Then the alpha power of all dipoles of region of interest, left and right auditory cortices, were averaged for regulation and pink noise phases. In the next step non-parametric Wilcoxon signed rank test was used, then the *p*-values were corrected for multiple comparisons using the Benjamini & Hochberg method [144].

5.2.4 Behavioral data analysis

Firstly, regardless of group type, THI, STAI-T, BDI, PSQI and WHO scores (pre, post) were analyzed using Wilcoxon signed rank test which is a nonparametric test for two populations when the observations are paired, then in determining significance multiple comparisons the Benjamini & Hochberg method [144] was applied to correct for the *p*-values. Secondly for each type A (A1and A2) and type B, the same analysis was done using Wilcoxon signed rank test[153], to examine whether there are any significant, between pre and post behavioral assessments. Lastly, to compare the two types, A and B Kruskal-Wallis test[153] is applied as a nonparametric version of a classical one-way ANOVA, and an extension of the Wilcoxon rank sum test to more than two groups.

5.2.5 Sensor level and source level analysis on resting state EEG

The absolute power spectral density for each channel and EEG segment was computed by using Multitaper fast Fourier transform with 3 tapers[154]. Power spectral analysis was performed using the Fieldtrip toolbox[155]. For depicting the power spectrum for three visits the frequency range (1-48Hz) calculated for every trial and each subject and channel, the power spectrum was calculated and averaged over all channels. Finally , the frequency spectra for these three visits were depicted.

To compare the alpha activity alteration across these three time points, one of the source localization method was applied on these three resting state EEG data. Firstly we calculated the volume conduction model of the template MRI of Fieldtrip data set on the basis of the boundary element method[143]. Then Standardized low-resolution brain electromagnetic tomography (sLORETA)[128] was used to estimate source activity in alpha band(8-13 Hz). To compare voxel-by-voxel the current density amplitudes of the alpha

band, the permutation multiple comparison t-sum approach was used ,which accounts for multiple comparison[156].

5.3 Results

The selected channels (excluding EOG channels) for neurofeedback session for each patient are shown in table 5-3. Two references channels (green) and 8-12 optimal channels reflecting mostly auditory activity (blue) and 3-8 channels indicating visual activity (red). It can be seen that our localization procedure is precise.

Table 5-3: Reference channels (green), Optimal channels (blue), Visual activity channels (red)

Patients' Number	
2	TP9-TP10-FC3-FC1-FC2-FC4-FC6-C3-C1-Cz-C2-C4-PO7-O1-Oz-O2
3	TP9-TP10-FC3-FC1-FC2-FC4-C3-C1-Cz-C2-C4-PO3-POz-PO4-O1-Oz-O2
4	TP9-TP10-FC1-FC2-FC4-C5-C3-C1-Cz-C2-PO3-POz-PO4-O1-Oz-O2
6	TP9-TP10-F1-Fz-F2-FC3-FC1-C3-C1-Cz-PO3-POz-PO4
7	TP9-TP10-F7-F8-FC5-FC6-C5-C6-CP5-CP6-PO3-POz-PO4-O1-Oz-O2
9	TP9-TP10-FC5-FC3-FC1-C3-C1-Cz-C2-C4-CPz- PO3-POz-PO4-O1-Oz-O2
10	TP9-TP10-AF3-F3-F1-Fz-F2-TP7-CPz-P1-P2- PO3-POz-PO4-O1-Oz-O2
11	TP9-TP10-F1-Fz-F4-FC5-FC3-FC1-C3-C1-CP1-CPz-CP2-CP4- PO3-POz-PO4-O1-Oz-O2
13	TP9-TP10-FC3-FC1-FC2-C1-Cz-C2-C4-CP1-CPz-CP2-PO3-POz-PO4-O1-Oz-O2
15	TP9-TP10-C3-C1-Cz-C2-C4-CP2-CP4- PO3-POz-PO4-O1-Oz-O2
16	TP9-TP10-FC2-FC4-C3-C1-Cz-C2-C4-CP3-CP1-CPz-PO3-POz-PO4-O1-Oz-O2
17	TP9-TP10-C3-C1-Cz-C2-C4-CP2-CP4-PO3-POz-PO4-O1-Oz-O2

The pattern of changing alpha across visits for each patient is shown in Figure 5-2. Table 5-4: shows p-values and slope and r-squared for each patients of his/her own learning curve.

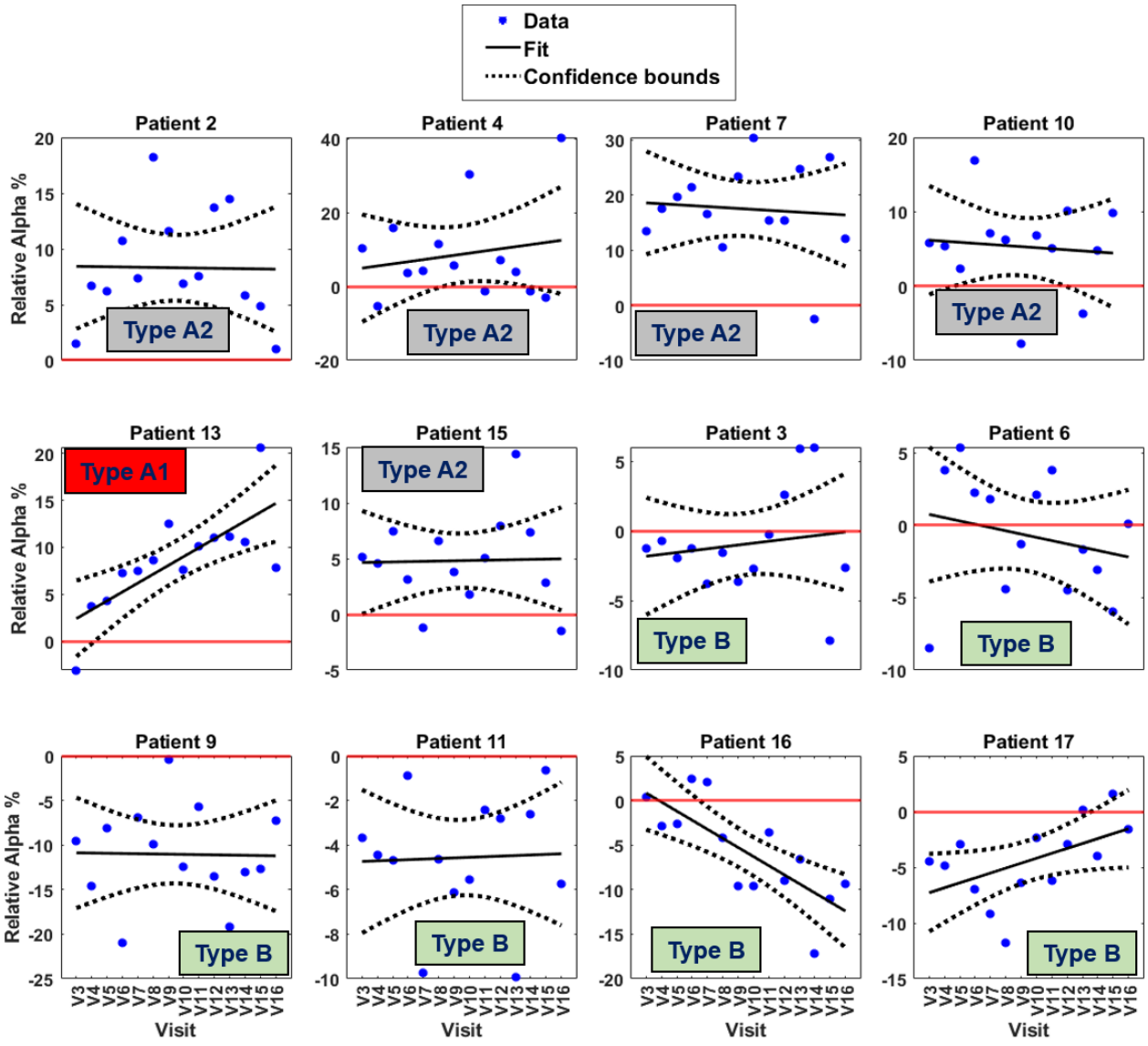

Figure 5-2: Change of filtered alpha power across 14 training visits for three types of subjects, Type A1: keeps the alpha filtered power of the training visits significantly above baseline (red) and the learning curve has a positive trend with a significant p-value, Type A2: the same characteristic as TypeA1 but the learning curve does not have a positive trend with a significant p-value, Type B: cannot keep the alpha filtered power of the training visits significantly above baseline.

The result of transfer runs for the learner group, shows no significant difference across training visits (visits number 8 to 14) . It can be concluded that none of the learner participants have transferred the learning skills to the daily life. Therefore, more training sessions in the natural environment would have been necessary.

The result of pink noise demonstrates that only two patients have a drop of alpha activity in auditory areas during the pink noise phase compared to the regulation phase (Table 5-5:).

Table 5-4: Corrected p-value (alpha feedback trials compares to baseline), Slope of the learning curve, p-value (learning curve) and R-Squared for three types of subject's Blue color indicates Type A1, green Type A2 and red Type B respectively.

Patient Number	Patient Type	*P*-value (baseline comparison)	Slope of the learning curve	*P*-value (learning curve)	R-Squared
2	Type A2	0.0008	0.0311	0.9241	0.0007
4	Type A2	0.0031	-0.3183	0.5126	0.0365
7	Type A2	0.0007	0.1387	0.8117	0.0049
10	Type A2	0.0034	-0.1044	0.8068	0.0051
13	Type A1	0.0004	0.4972	0.0259	0.3496
15	Type A2	0.0019	-0.0107	0.9557	0.0002
3	Type B	0.2345	0.3298	0.2238	0.1205
6	Type B	0.8691	-0.1769	0.6550	0.0171
9	Type B	0.9812	1.0429	0.0144	0.4048
11	Type B	0.9585	0.0807	0.6422	0.0185
16	Type B	0.9865	-0.5534	0.0727	0.2437
17	Type B	0.0912	1.3524	0.0098	0.6176

Table 5-5: Corrected p-value (alpha feedback in regulation phase compares to pink noise). Significant values in blue.

Patient Number	Patient Type	*P*-value (regulation-pink noise comparison)
2	Type A2	0.853
4	Type A2	0.029
7	Type A2	0.356
10	Type A2	0.344
13	Type A1	0.456
15	Type A2	0.028
3	Type B	0.756
6	Type B	0.569
9	Type B	0.912
11	Type B	0.156
16	Type B	0.345
17	Type B	0.455

The results of pre- and post- behavioral assessments of all 12 patients (visit 1 and visit 17) , for five different questioners (THI, STAI-T, BDI, PSQI, WHO) is shown in Figure 5-3. The results of pre- and post-behavioral assessments (visit 1 and visit 17), for five

different questionnaires (THI, STAI-T, BDI, PSQI, WHO) for the two types (Type A and Type B) of patients showed **no significant** between these two patient types But both patients' types shows only significant reduction in THI scores (Tinnitus Score), *p*-value after correction type A, 0.0331 and type B, 0.0311.

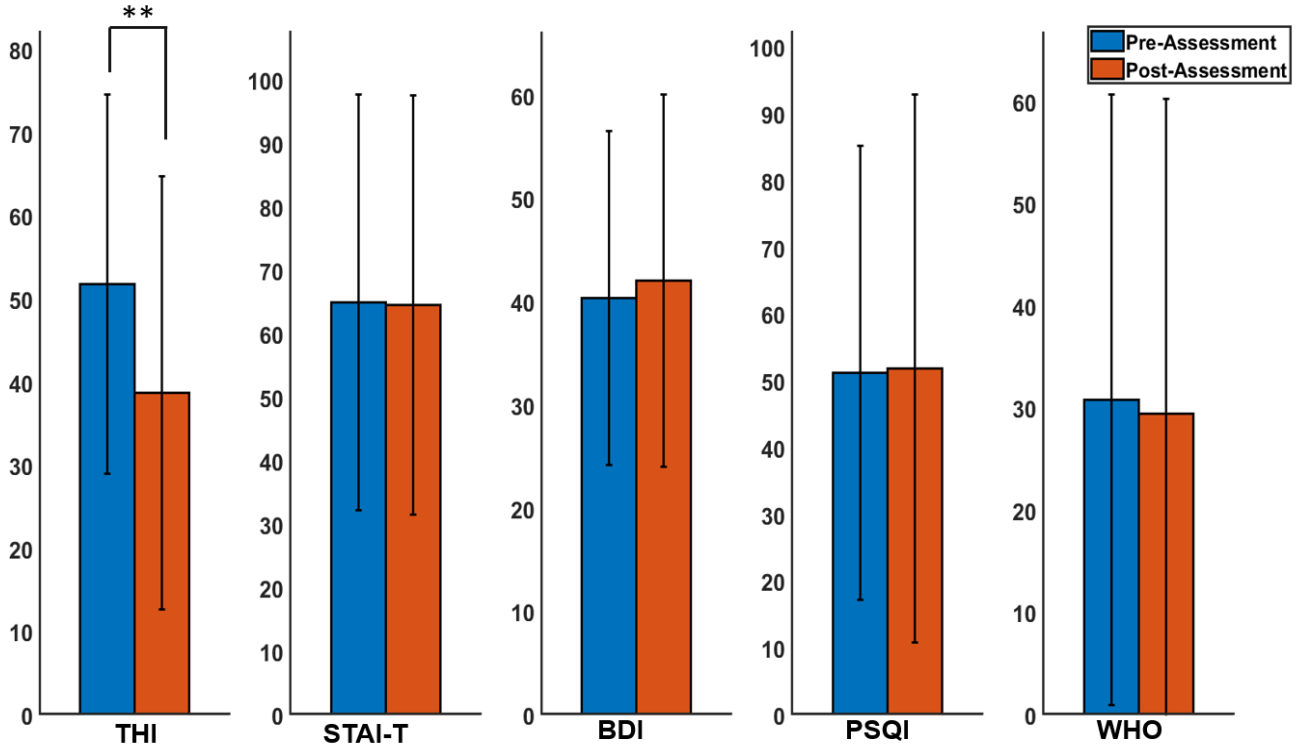

Figure 5-3: Summary Scores of five different questionnaires for all 12 patients (THI; Tinnitus Handicap Inventory; BDI = Beck Depression Inventory; STAI-T = State and Trait Anxiety Inventory – Trait; PSQI = Pittsburgh Sleep Quality Index) in two time points, one week before localizer visit and one week after the last resting state. (*p < .05; **p < .01; ***p < .001)

The histogram of similarity index indicating the similarity with auditory cortex activity for all 12 patients for all training visits.

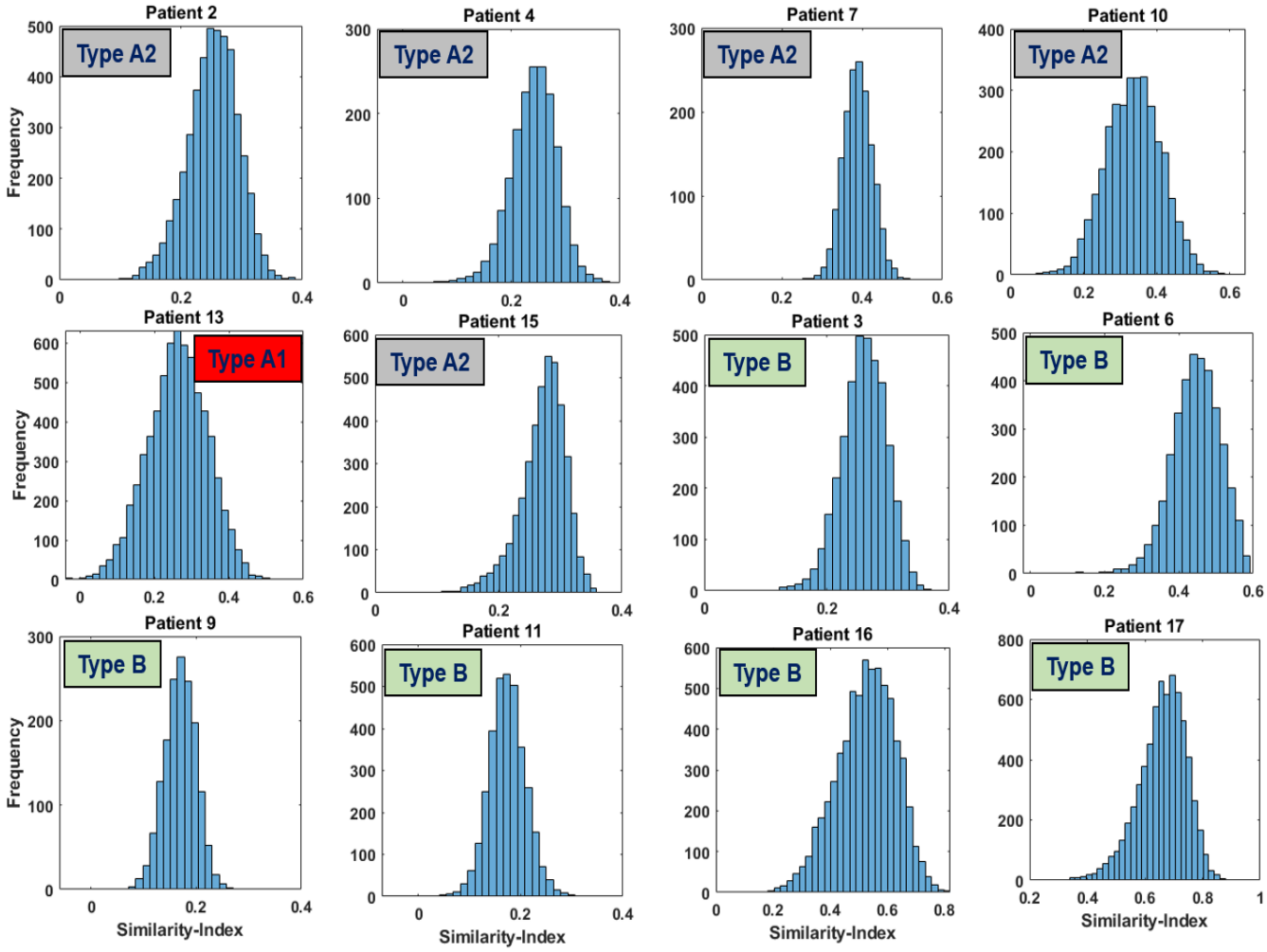

Figure 5-4. The representation of the distribution of similarity indexes which is averaged only across segments (each 1 second) for each trial and then mapped as a histogram.

Figure 5-5 shows Alpha power over training time on sensor level for three resting state visits for the patient type (A1-A2) and patient the type (B2).

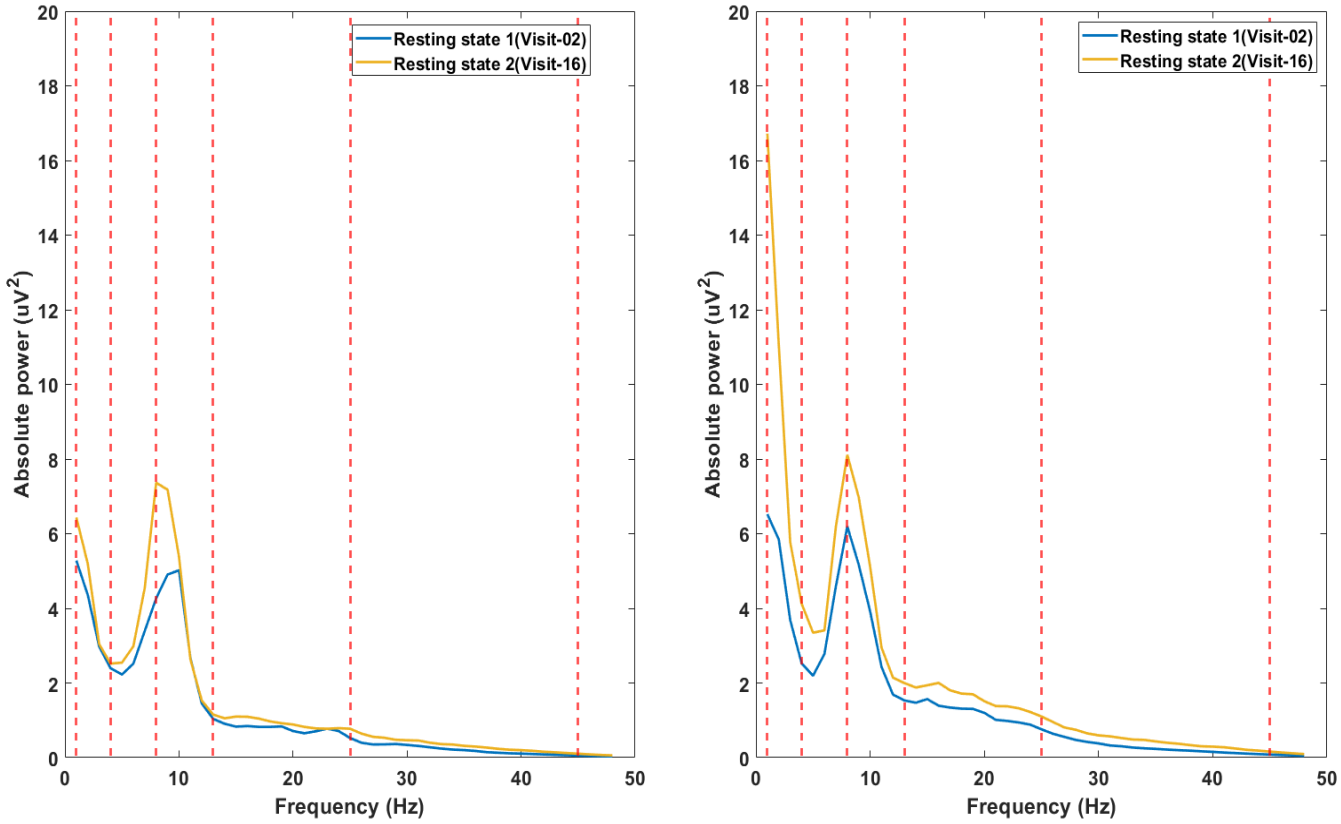

Figure 5-5. Frequency power spectrum for two resting visits which is the result of Fourier transformation, averaged across channels and then subjects for patient type(A1-A2) left panel and patient type(B) right panel. Two consecutive dashed redlines indicates a specific band power, delta(1-4), beta(4-8), alpha(8-13), beta(13-25) and gamma(25-45). Despite lack of significance the increase in alpha during resting is visible in both groups.

The result of source level of resting visits showed no significant changes in region of interests, auditory left and right and temporal left and right for the all 12 participants and also both subject type (A1-A2) and subject type(B).

5.4 Discussion

The main goal of this preliminary exploratory study was to apply a novel neurofeedback paradigm reinforcing auditory cortex activity in patients with chronic tinnitus in an attempt to reduce tinnitus related distress via normalization of alpha brain activity. This was pursued by up-regulation of alpha activity, i.e. the normal resting state activity of the

auditory cortex, which is decreased in tinnitus distress and by assessing the effect of neurofeedback training on neural activity of the target region. This study revealed many interesting results which are discussed in detail here.

5.4.1 Channels reflecting auditory and visual activity

The result of the first localizer visit shows for each participant two set of channels which reflect auditory and visual activities. As it can be seen in table 5-3, the auditory channels for all the 12 participants are located dominantly in three different areas, Fronto-central (FC), Central (C) and Centro-parietal(CP). Based on previous studies it has been shown that the orientation of the auditory dipoles points towards to the central regions of the brain which is with the location of the selected channels [141,142]. The visual channels which are located at the occipital lobes are correctly localized. Therefore, it can be concluded that all participants had the same response to the presented auditory stimulus in the first localizer, though the degree of tinnitus severity and hearing deficit among them was not the same.

5.4.2 Learner and non-learner participants

Learning consists of **stable changes** in behavior caused by experience. These **stable changes** have to be quantified using mathematical models. Thus, two approaches are applied in this thesis:

1. The neurofeedback outcomes can be compared to the baseline. The null hypothesis is that there is no statistically significant difference between the neurofeedback outcomes and the baseline and the alternative hypothesis is a statistically significant difference between the neurofeedback outcomes and the baseline.
2. The neurofeedback outcome across time has a positive significant trend which its parameters are statistically significant, like a positive linear model with significant slope.

As in the neurofeedback treatment paradigm, the main objective is to up- or down-regulate a specific brain activity from a targeted brain region, therefore in this thesis both criteria are used in chronological manner. The relative alpha power for all 14 training

sessions was tested against zero with a non-parametric test. The results, Figure 5-2, shows that only 6 participants could fulfill this criterion. Furthermore, only one participant of these 6 learners, could increase alpha level linearly across the training sessions. The other five subjects may have improved their learning skills, if they could have been given more training sessions. But the other 6 remaining subjects, non-learner, they could not fulfill the first criteria, therefore they could be not be considered as learner. The participant who could reach above baseline across these 14 sessions, has developed a skill. Although subjects' type-**A2** could not increase the alpha level in a linear model across the sessions, this does not mean randomness which might be regarded as non-learning, because they fulfilled the first criteria. One of the crucial points in both approaches is the number of the training sessions. As both approaches are dependent on the number of the training sessions, more training sessions could change the participant assignments . For instance, subject number 17 has positive trends, but he could not reach above the baseline. If the same subject were more training session given, he could have reached above the baseline, therefore he would have been assigned to type A1.

The result of transfer runs shows that even the learner group could not transfer the learning skill to the daily life environment.

The significant drop of alpha activity in the auditory cortex during pink noise phase compare to regulation phase was observed only in two patients. The reasons we could not see the same pattern in the other patients might be:

1) As the length of the pink noise phase was 8-10 seconds the habitation processes might after some seconds of pink noise presentation occur which results in no change in alpha level in the auditory cortex.

2) As the patients exposed to pink noise 20 times per each run, each 8-10 seconds, the habitation processes might occur after some trails.

3) During the noise- phase the patient was instructed to relax, and no task was performed. Since alpha waves are linked with relaxed mental states, this pink noise phase might in fact increase the global alpha level which in turn could increase the alpha activity in auditory cortex.

5.4.3 The Similarity Index

The similarity index is an indirect measure of alpha activity at the source level of non-auditory cortex. Closer to 1 indicates less activation of non- auditory sources and mor auditory activity. Figure 5-4 shows the upper range of the similarity index for all 14 training sessions for most of the patients is close to 0.5 and in some patients is close to 0.7. This demonstrates that the feedback given to the patients was not completely originating from auditory cortex and is contaminated with alpha activity of non-auditory regions.

5.4.4 Changes of questionnaires scores and alpha in auditory cortex

Only the behavioral questionnaires scores (THI) was significantly dropped in both learner and non-learner groups. But there was no significant change in alpha level of auditory cortex in both groups. The reason for this could be a low range of similarity indices during training sessions. The conclusion we can obtain from this part, would be that the significant changes in THI score is not a consequence of changing in alpha level in auditory areas or that the non-significant alpha changes are already sufficient to affect the subjective Tinnitus.

Chapter 6

6 General Discussion

6.1.1 Objectives and findings of the research

Chronic tinnitus is affecting 10-15% of people typically for many decades, with increasing prevalence during aging. Multiple therapy forms for tinnitus exist (including behavioral therapy, external white noise stimulation, various kinds of alternative approaches), but up to now no accepted successful treatment exists. Previously, it was shown that voluntary control of the de-activation of the auditory cortex can be learned by means of real-time Functional Magnetic Resonance Imaging (fMRI) neurofeedback, and that it may alleviate tinnitus symptoms. The same seems to be true for learned increase of alpha Electroencephalogram (EEG) activity localized in the auditory cortex. Given the high prevalence of chronic tinnitus, its significant burden for affected individuals, and given the absence of a generally effective therapy, neurofeedback training for tinnitus has the potential to become a broad clinical application. Therefore, The aims of this research could be chronologically divided into three parts: 1) to design and implement an EEG novel neurofeedback paradigm which contains a novel spatial filter for monitoring of auditory cortex activity, tactile and visual stimulation to suppress somatosensory and visual alpha and pink noise stimulus for relieving the tinnitus suffering and suppress alpha in the non-regulation phase which could facilitate up-regulation of alpha activity. 2) using the same system for neurofeedback in chronic tinnitus patients to normalize their low alpha activity in auditory cortex.

Some very interesting consequences emerge from this research. 1) Alpha auditory activity can be monitored from EEG sensor level which does not involve any problems of inverse solution of the standard source localization methods. 2) Up-regulation of alpha auditory activity seems to be difficult for patients, because half of the recruited participants were

not able to regulate this activity. 3) The reduction of main tinnitus questionnaire scores (THI) was achievable, though alpha in auditory areas could not be significantly changed across training sessions. Whether this can be regarded as a placebo response or a consequence of the small but existing effort to increase auditory alpha cannot be decided on the basis of these data.

6.1.2 Relationship with previous research

According to the volume conduction, origin of EEG it is uncontrolled where in a trainee's brain the
EEG changes due to classical neurofeedback training are generated. This might vary from person to person and this variability may be one reason for the moderate therapeutic effects of neurofeedback training[157]. Therefore, real-time source localization of EEG or MEG have been developed in recent years[158-160]. All these online source localization methods encounter two problems: the low signal-to-noise ratio (SNR) and the limited time available for online computations. Since each 500 milliseconds or 1 second the given feedback must be updated, the segment of the data may be unreliably short to be processed in an inverse EEG solution. On these grounds we designed a method which circumvent the above issues. The method uses the localizer data which it manifests the primary auditory cortex activity. Having the localizer data, a spatial filter was designed which could be applied in real-time procedures for the EEG-neurofeedback paradigm. To our knowledge, this way monitoring of an activity of a specific region in real-time has not been yet reported. Hence, this kind of source monitoring could enable learning of spatially targeted self-control of cortical activity.

6.1.3 Limitations of the research

Regarding the designed spatial filter, it can be applied only for monitoring of auditory area not any other brain regions. Hence, the other non-auditory EEG-neurofeedback paradigms could not use this method. The effect of tactile stimulations or visual stimulation on mu-rhythm and visual alpha was not evaluated but only hypothesized. The experimental and statistical comparison between a neurofeedback-condition with simultaneous visual-somatosensory stimulation during the neurofeedback training trials

and a neurofeedback condition without simultaneous stimulation would indeed be highly interesting and of great scientific and clinical value but needs an extensive and large clinical trial involving many patients. In this experiment only the first and most important steps could be realized.

There are two major limitations of the clinical trial : 1) As a proof of concept investigation the study evidently has a small sample size but extensive training times, 2) to examine whether the neurofeedback outcomes result from neurofeedback-specific effects, the experimental paradigm must include one or more control groups or conditions to rule out non-specific effects (e.g. sensory stimulation, placebo).

6.1.4 Problems arising during the research

1) At the initiation of this research, it was planned to implement another real-time source localization method. However, after six months of testing the new method described here Was adapted because the mathematical performance of the previously developed method was not satisfactory.

2) After finishing the first phase of this project, one of the researchers involved in many parts of the project left the study because of a new position in industry . Consequently, a new researcher with approximately the same scientific qualifications have been hired and trained which in turn interrupted the smooth running of the project for at least three months.

3) The project was initiated at the University of Tübingen - Germany, after one and half year the project leader decided to collaborate with an additional project on tinnitus rehabilitation strategies which was conducted in Geneva – Switzerland from 2013 to 2016. On the ground of this collaboration, some changes in our inclusion and exclusion criteria have been made resulting in losing many potential participants and leading to the small sample size.

4) Training sessions required 15 weekly basis visits and the participants attended these sessions during weekdays. As many of registered participants could not arrange these many sessions, they left the study before starting training sessions.

6.1.5 Implications of the findings and recommendations

The method proposed can be used and implemented to monitor auditory cortex activity in a real-time application. Findings from the first study (chapter 5) shows that at least when primary auditory cortex activity would be targeted, the measurement of its activity is feasible using the similarity method. It is also possible to stimulate other brain areas with specific visual or tactile stimuli in order to design a special filter.

Distinction between learner and non-learner is still a very controversial topic. Having a positive liner trend as an indication of learning is only one possibility as a mathematical representation of neurofeedback output. On the other side, if learning is a stable change of behavior, this linear learning curve also must manifest itself correlated in the visit based-given questionnaires, meaning that there should be a strong correlation between targeted brain plasticity and observable behavior. Otherwise, the pure neurofeedback learning curve is not an indication of targeted brain region changes.

The question " why do some participants based on one or another learning definition cannot learn to control neurofeedback signal?" is still an enigma in the neurofeedback literature. Systematic investigation of the reasons, why some subject could learn, and some subject is not vital in neurofeedback. This helps both subjects and researchers to get better picture of the learning procedures and build up a more versatile neurofeedback system.

Sample sizes in neurofeedback need to be very large, minimum sixty subjects: firstly, the large sample size could allow to compare learner and non-learner, secondly, as the number of training sessions are usually more than 8 or 10, some of the subjects might not finish the whole neurofeedback time plan, therefore, it is crucial to not have a small sample size at the end of study.

Another important point is that in each neurofeedback visit, the patient should be questioned to observe the behavioral outcome after each visit, also to quantify the motivation, stress, sleep and expectancy for success levels due to the effects of all these factors on neurofeedback outputs and to control for placebo responses.

In most of the neurofeedback studies the patients are not instructed any specific strategy[161]. Motor-imagery-assisted brain-computer interface (BCI) is the exception. The reported mental strategies and the subsequent categorization of them are also essential part of the neurofeedback[162]. However, no clear picture of a suitable strategy emerged.

Overall, the present clinical experiment constitutes the first attempt to follow the dominant theory of the origin of chronic tinnitus with a specific and differential treatment approach by training severe tinnitus sufferers over an extensive time period to increase neuronal inhibition of the tinnitus brain network. We developed a new and innovative online neurofeedback training to increase auditory cortex inhibition by increasing localized alpha activity of both auditory cortices. The limited effectivity of this new approach may have many reasons difficult to isolate in a clinical trial whose aim was to demonstrate that such an approach is possible at all.

The main limitation may consist in the validity of the underlying neurophysiological theory: as we have illustrated in our theoretical discussion the neuroplastic model of increased neuronal activity in the auditory central system is by no means a proven and replicated concept but still in a state of emergence and need intensive basic animal and human research. A purely subcortical and more peripheral location of the deficit would make a centrally focused approach obsolete.

Still ,most facts point to a critical involvement of the cortical system in the genesis of the disorder. Even then, the inhibitory “power” of a localized alpha wave increase may not be sufficient to block the overactivation in the auditory system. Or, as already indicated above, the training procedure was too short to automatize the necessary skill, or the learning condition stood in the way of an automatic skill applicable in the natural environment.

We still urge the field to replicate and extend our findings and the chosen approach with a large sample of severely affected individuals in the direction discussed above.

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