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EVALUATING THE PROGNOSIS VALUE OF INTENSITY VARIABILITY IN TINNITUS TREATMENT WITH ACOUSTIC STIMULATION DURING SLEEP

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TABLE OF CONTENTS

1. Abstract

Several strategies of acoustic stimulation have been developed for the treatment of tinnitus during the last decades. In particular, the use of a stimulation protocol individualized for each patient, that reproduces the tinnitus frequency content and volume, and is applied during sleep, has been shown to be very effective (e.g., around 14.5 dB of stable volume reduction). A group of researchers and clinicians in Uruguay developed a system which allows to create a personalized acoustic receipt that is loaded on a mobile device to run the acoustic stimulation during sleep. This acoustic stimulation treatment is divided into three phases: adaptation, continuous stimulation, and detachment. The focus of this thesis work was the time series formed by the successive volume annotations done during one year by 18 tinnitus patients. We assessed the hypothesis that the variability of the volumes recorded during the adaptation phase has a prognosis value on the outcome of the treatment. The outcome is measured as the volume reduction achieved at the end of the continuous stimulation phase in comparison with the volume at the start of the treatment (as recorded in the acoustic receipt). To test this hypothesis, we performed a linear regression between several statistical indexes extracted from the volume variability during the adaptation phase and the volume reduction. Using an F test, we demonstrated the existence of a significant increasing trend in the regression line for some of the variability indexes tested (standard deviation, standard error, range and skew up). The best predictor of the treatment outcome was the skew up from the median of the distribution. This finding is discussed in the context of known neuroplasticity changes induced by acoustic stimulation. It appears that a larger variability in the tinnitus volume during the adaptation phase of the treatment can be indicative of a brain with more capacity for synaptic plasticity, which is key to achieve a reduction of the tinnitus.

2. Introduction

2.1 A brief overview of the auditory system

To comprehend the relation of peripheral receptor and the auditory pathways with tinnitus, we present a summary of the different components involved in sound perception.

2.1.1 Sound

A sound consists of vibrations that travel through air or another medium, which can be heard by a person's auditory system. Sound waves transmit energy through the vibration of molecules, alternating increases and decreases in pressure (i.e., compressions and rarefactions). The travelling speed in air is approximatively 761 mph. The frequency of a sound, measured in Hertz (Hz), defines the number of alternating compressions and rarefactions per second. The human ear can hear sounds with frequencies of between 20 to 20,000 Hz. The auditory system has the capability to transduce pressure changes in the air into action potentials and sound perception (Kandel et al, 2013).

2.1.2 The peripheral receptor of the auditory system (Figure A)

The peripheral receptor is divided in three different structures:

a) *The external ear*

The first and foremost structure involved in sound perception is the external ear and is made up of the auricle and the external auditory canal. In it, sound waves (alternating compressions and rarefactions) are directed towards the ear drum. The external ear has a physical structure that

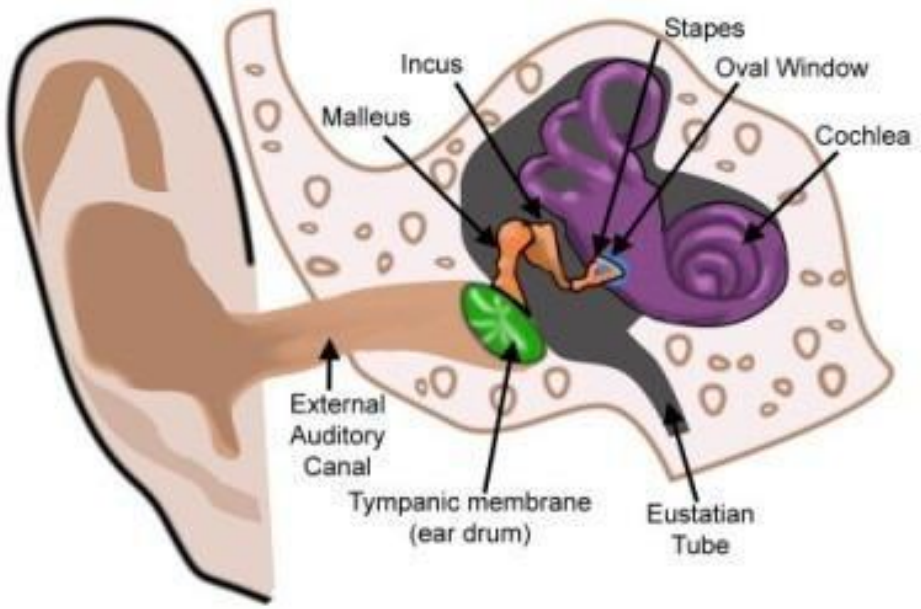
passively and selectively amplifies the frequencies involved in intraspecies communication (Kandel et al, 2013).

b) The middle ear

The middle ear is the portion of the ear medial to the eardrum, and distal to the oval window of the cochlea . It contains three ossicles - malleus, incus, and stapes-, which transfer the vibrations of the eardrum into waves in the fluid and membranes of the inner ear. The hollow space of the middle ear is also known as the tympanic cavity and is surrounded by the tympanic part of the temporal bone. The auditory tube (also known as the Eustachian tube) joins the tympanic cavity with the nasal cavity (nasopharynx), allowing pressure to equalize between the middle ear and throat. The primary function of the middle ear is to transfer and amplify acoustic energy from compression waves in air to fluid–membrane waves within the cochlea (Kandel et al, 2013).

c) The inner ear

The inner ear is the innermost part of the ear and is mainly responsible for sound detection and balance. It consists of the bony labyrinth, a hollow cavity in the temporal bone of the skull comprising two main functional parts, the cochlea (dedicated to hearing) and the vestibular system (dedicated to balance)(Kandel et al, 2013).



2.1.3 The cochlea

The cochlea is the receptor organ of the auditory system. It is a spiraled, hollow, conical chamber of bone, in which waves propagate from the base (near the middle ear and the oval window) to the apex (the top or center of the spiral) (Figure B). The spiral canal of the cochlea is approximately 30 mm long and makes $2\frac{3}{4}$ turns. The cochlear structures include:

- Three *scalae* or chambers:
    - the vestibular duct or *scala vestibuli* (containing perilymph), which lies superior to the cochlear duct and abuts the oval window.

Figure A. A cross-section of the peripheral receptor structures, including the outer, middle, and inner ear with labelled substructures (Peterson et al, 2021).

    - the cochlear duct or *scala media* (containing endolymph) a region of high potassium ion concentration that the stereocilia of the hair cells project into.
- The basilar membrane, a main structural element that separates the cochlear duct from the tympanic duct and determines the mechanical wave propagation properties of the cochlear partition. On top of the basilar membrane, the sensory epithelium or organ of Corti is located (Kandel et al, 2013).

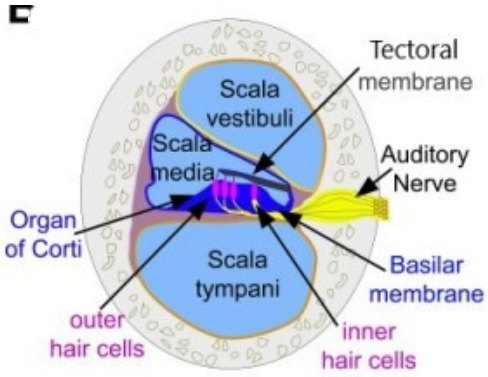


Figure B. A cross-section of the cochlea, with labelled structures involved in the auditory pathway, including the organ of Corti (Peterson et al, 2021).

2.1.4 The organ of Corti

The Organ of Corti, is a sensory epithelium responsible for transducing mechanical energy into electrical impulses. It consists of sensory hair cells located inside the scala media chamber, on top of the basilar membrane. The hair cells are epithelial cells topped with hair-like structures called stereocilia. The cochlea receives sound in the form of vibrations, which cause the stereocilia to move opening potassium ion channels. The flow of potassium ions into the hair cell generates changes in its resting potential, which are synaptically transmitted to the terminals of the auditory nerve (Figure B). As a result, the external sound information is converted into electrical impulses, allowing the auditory nerves to carry the information to the auditory cortex for sound identification and perception (Kandel et al, 2013).

The receptor organ contains inner and outer hair cells. The inner hair cells are the ones responsible for the mechanoelectrical transduction. The mechanical properties of the basilar membrane allow their different parts to vibrate in resonance with different sound frequencies, with the basal part tuned to higher tones and the apical part to lower tones. The outer hair cells amplify the movements of the basilar membrane through contractions of a protein called pristin located in its cytoplasm (Kandel et al, 2013). Pristin forms a network that shortens the length of the cell in response to changes in voltage generated by the entry of potassium ions. Those contractions move the basilar membrane, thus amplifying the response of the inner hair cells (Purves et al, 2001).

2.1.5 Tonotopic coding through labelled lines at the basilar membrane

Both outer and inner hair cells are arranged in a tonotopic map organization determined by their position in the cochlear spiral. In this way, different parts of the cochlea spiral are sensitive to

specific frequencies of sound and activate specific fibres of the auditory nerve creating the so-called labelled line code. In this type of neural coding, each frequency of sound will elicit action potentials in specific fibres of the nerve (Kandel et al, 2013).

2.1.6 Central Auditory System

The Afferent auditory pathway

Information from the peripheral auditory system reaches central auditory nuclei via the auditory nerve. From the auditory nuclei the information is transmitted upwards till it reaches the cortex where perception occurs. These nuclei include 1) cochlear nucleus, 2) superior olivary nuclei, 3) lateral lemniscus, 4) inferior colliculus, and 5) medial geniculate nuclei.

Auditory information ascends through the auditory nerve and makes the first synapse relay within the cochlear nucleus. Most of the auditory information is then transmitted through crossing fibers into the superior olivary complex. In this step most of the fibres decussate, meaning that they mainly innervate the contralateral olive, before ascending to the inferior colliculus and the medial geniculate nucleus. The information is decoded and integrated by each relay nucleus of the pathway and finally projected to the auditory cortex, at the temporal lobe, where a conscious perception is generated (Figure C). It is of note that a significant number of neurons within the auditory system have crossing fibers at every level of the auditory system. This is likely due to the need for both ipsilateral and contralateral information for many aspects of auditory processing. Therefore, all levels of the central auditory system receive and process information from both the ipsilateral and contralateral sides.

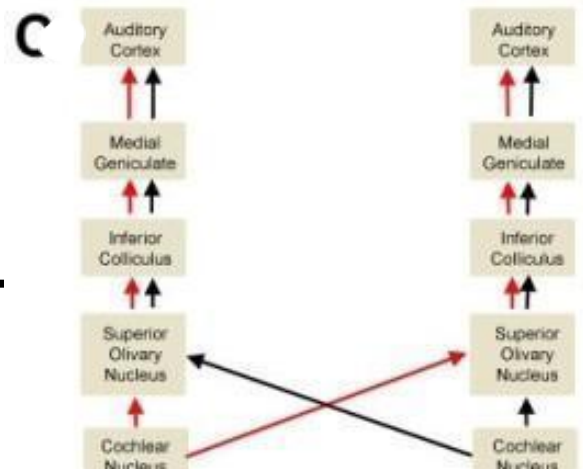


Figure C. An overview of the afferent pathway from the cochlea to the auditory cortex, indicated in red and black arrows (Peterson et al, 2021).

Different aspects of environmental sounds (e.g., attenuation: how loud the sound is, location in space, and frequency) are processed in each of the central auditory areas. Most of the auditory nuclei throughout the brain are tonotopically arranged. In this way, auditory signals ascending to the cortex can preserve the frequency information from the environment.

The Efferent auditory pathway

It was once thought that auditory processing was a simple relay from the environmental signals up to the cortex. We now know that there is a significant descending system of circuits within the auditory system that helps to modulate auditory processing at every level. The auditory cortex has bilateral direct projections back to the inferior colliculus, superior olivary complex, and cochlear nucleus. These circuits contact neurons in these nuclei that project to every level of the central auditory system. The olivocochlear efferent system innervates mainly the external ciliary cells through the vestibulocochlear nerve and is particularly important in the generation of subjective tinnitus. Connections between descending, ascending, and crossing fibers make the auditory system highly interconnected. These descending circuits help to modulate auditory attention based on the relevance, attention, learned behaviours, and emotional state of an individual. Such higher-order functions originate from many regions of the brain (e.g., prefrontal cortex, hippocampus, nucleus basalis of Meynert, and limbic circuits) that have either direct and indirect connections with each other and auditory cortex (Figure D).

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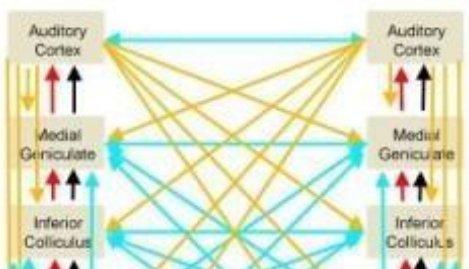

Figure D. An overview of both afferent and efferent auditory pathways involved in central auditory processing. (Afferent pathway: red and black arrows. Efferent pathway: orange arrows. Interconnections between structures: blue arrows) (Peterson et al, 2021).

2.2 Tinnitus

Affecting approximately 10-15% of the world's population, tinnitus is an auditory symptom where the patient perceives sounds in the absence of external auditory stimuli. From the affected population, approximately 1-2% of the patients experienced severe impairment of the quality of life and the ability to work (Han et al, 2009). There are two types of tinnitus, which are known as subjective and objective.

2.2.1 Objective tinnitus

Objective tinnitus is a rare event, in which the sound is audible by an external observer. In some cases, it may be a pulsating hum, caused by turbulences through the carotid artery. Highly vascular middle ear tumours, such as jugular glomus tumours, and dural arteriovenous malformations can also cause objective tinnitus (Grossan and Peterson, 2022).
In those cases, the perception is created by a real sound of pathological origin.

2.2.2 Subjective tinnitus

In most cases, the tinnitus is classified as subjective, and its main characteristic is that it can only be heard by the patient. There are a broad range of possible causes for subjective tinnitus, but only in 3-4% of cases an underlined pathology is detected. Within those pathologies, we can mention the following: multiple sclerosis, brain tumours, high blood pressure, metabolic disorders, neurovascular conflicts, ear wax plugs, middle ear pathology, etc. The other 96-97% of

the cases, in which the cause cannot be established, are classified as subjective idiopathic tinnitus (Atik, 2014).

2.2.3 Characteristics of subjective idiopathic tinnitus

The sounds associated with most cases of subjective tinnitus have been described as resembling crickets, wind, water flow, steel grinding, steam escaping, neon lights buzzing and engines running. The sound may or may not be sustained, it may build up or begin abruptly and is best perceived in quiet situations, undisturbed by background noises. An identification and characterisation of 5 different types of tinnitus sounds was carried out by the team of researchers at Uruguay: single pure tone, combinations of pure tones, broadband noise, combination of broadband noise with a single pure tone and white noise. A particular type of combination of pure tones resembling the sound of a 'cricket' sound was referred by many patients (Drexler et al, 2016; Kostarakos and Römer, 2010).

2.2.4 Etiopathology of subjective idiopathic tinnitus

The postulated trigger for tinnitus pathogenesis is the disregulation of the central auditory processing induced by altered cochlear inputs (Jastreboff, 1990). In most cases, damage to outer hair cells in particular cochlear regions leads to a reduction of spontaneous activity in related nerve fibers, and an imbalance in excitatory and inhibitory networks (Eggermont & Roberts, 2004), which leads to hypersensitivity and hyperactivity in the neurons involved. Hyperactivity may also be the result of reorganization of the cortical tonotopic map after cochlear damage, which induces a release from efferent inhibition at the characteristic frequencies that lose cortical representation (Robertson & Irvine, 1989; Rauschecker, 1999; Eggermont & Komiya, 2000; Noreña & Eggermont, 2003). Increase in spontaneous firing rate has been found at different levels of the auditory pathway: the dorsal cochlear nucleus (Brozoski et al, 2002), inferior colliculus (Mulders & Robertson, 2009), and auditory cortex (Noreña & Eggermont, 2005). Because the brain is not able to discern if this abnormal incoming flux of information is related to real environmental sound, a 'phantom sensation' (tinnitus) may be created (Jastreboff, 1990).

he one of the tinnitus, the inner ear Positron emission tomography (PET) scans and functional magnetic resonance imaging (fMRI) studies have shown that loss of cochlear input to central auditory neurons can create abnormal neural activity in the auditory cortex, causing tinnitus (Cai et al, 2019). Additionally, inhibition in the neural feedback circuits that help regulate and consolidate auditory memory can be lost in the auditory pathways and cortex. Disruption of these feedback loops can lead to the formation of alternate synaptic pathways, resulting in the abnormal information processing underlying tinnitus (Atik, 2014). Subjective idiopathic tinnitus may be the result of neural plasticity, and anomalies may develop because of decreased input from the ear, deprivation of sound stimulation, overstimulation or yet unknown factors (Jastebroff, 1990).

To summarise, the subjective idiopathic tinnitus may be the result of neural plasticity, and anomalies may develop because of decreased input from the ear, deprivation of sound stimulation, overstimulation or yet unknown factors (Jastebroff, 1990).

2.2.5 The edge theory

The so-called “edge theory” suggests that tinnitus can be the result of an increase in the inner ear´s hair cells activity of the edge regions of a cochlear lesion. When one part of the cochlea is damaged, the efferent system will tend to release inhibition in this part to increase the input coming from the affected tones. The efferent system innervates mainly the outer hair cells, which are primarily damaged in the lesion, so the functioning of the cochlear amplifier is affected to a greater or lesser degree (depending on the severity of the injury) in the frequencies that encoded by the damaged part. The idea behind the edge theory is that the tuning of the efferent system is not sharp enough to restrict the inhibition release to the lesion, and it also disinhibits areas on the edges of the lesion that are not damaged. The external hair cells in these edges will be abnormally released and as a result, the inner hair cell input from the edge areas of the cochlea gets excessive amplification, creating the tinnitus (Haider et al, 2018). Acoustic stimulation treatments performed using the frequency content of the tinnitus are intended to induce plasticity

changes able to reverse the previous misled plasticity changes that resulted from cochlear lesions. By stimulating with a frequency content matching t
ncrease their activity, even if the cochlear amplifier function was diminished by the injury (Han et al, 2019). Then, the disinhibition of the edges of the lesion coming from the efferent system eases, decreasing the tinnitus volume. cells i

2.2.6 Tonotopic cortical maps reorganization as cause of tinnitus

Misled plastic changes secondary to lesions can be present at several levels of the auditory pathway. For example, decreased inputs from the auditory nerve can cause tinnitus due to disinhibition of the dorsal cochlear nucleus, causing abnormal spontaneous activity. .
At the auditory cortex level, several studies in animals have demonstrated that cochlear damage resulting from ototoxic medications or exposure to loud noise, could lead to an increased amplitude of cortical evoked potentials (Henry et al, 2014; Eggermont & Roberts, 2004). A reorganisation of tonotopic maps in the primary auditory cortex takes place (Mühlnickel et al, 1998), because the area that received input from the damaged part of the cochlea gets deprived from its input. In this situation this cortical region will start responding to frequencies originally mapped by the edges of the damaged cochlear region. In other words, in an event of a cochlear lesion, cortical regions that were covering the lesioned area can reorganize or retune to respond to frequencies near the lesion edges, leading to an over-representation of these frequencies in the tonotopic cortical maps that causes the tinnitus and increases the amplitude of evoked potentials to certain tones. (Adjamian et al, 2014). A treatment with sound stimulation using the frequency content of the tinnitus can prevent the decay of activity in the cortical areas mapping the lesioned area, avoiding their reassignment to neighbouring frequencies that underlies the tinnitus. (Pawlak-Osińska et al, 2013). Again, we found here an example of a plastic change in response to a cochlear lesion that ends causing tinnitus and a restorative plastic change created by the acoustic stimulation treatment (König et al, 2006).

2.2.7 Neural fibers crosstalk

Another example of misled neural plasticity is the development of crosstalk between fibres of the auditory nerve. Cranial nerves are sensitive to compression at the root entry zone due to blood vessels or tumours, causing crosstalk by ephaptic coupling or by the formation of aberrant synaptic connections. The crosstalk theory postulates that in an event of damage in the auditory pathway, the discharges of individual auditory nerve fibres can be abnormally coupled. The crosstalk development causes abnormal activity in the auditory neurons that is misinterpreted as sound (Cardon et al, 2012).

2.2.8 Impact of tinnitus on quality of life

Patients with tinnitus can have a significant impact on quality of life. They may experience social isolation, lack of concentration, anxiety, depression, and sleep disturbances. In some persons the symptoms can lead to easily feeling lethargic and tired. As a result, tinnitus patients are likely to spend more energy just trying to live, work and socialize than it is the case in the general population.

When discussing the impact of tinnitus on quality of life, it is important to understand its correlation with hearing loss. Patients with tinnitus always have, to a greater or lesser degree, hearing loss. Also, the presence of tinnitus can make communicating with family and friends more difficult, which can interfere with socialization and make them more likely to avoid social situations. These social factors can affect the patient's ability to comply with tinnitus treatments (Bhatt et al, 2017).

2.3 Tinnitus and treatments

2.3.1 Subjective idiopathic tinnitus treatments

The treatment of idiopathic subjective tinnitus has always been a great medical challenge since a therapeutic protocol has not yet been found to eliminate it definitively. However, the greater

knowledge of the pathophysiology that triggers tinnitus (Jastreboff, 1990; Eggermont and Roberts, 2004), identifying it as an error in auditory processing at the level of the central nervous system, has made therapeutic protocols evolve to more rational and efficient schemes that allow obtaining substantial changes in the intensity of tinnitus with the consequent improvement in the quality of life of the patient.

Tinnitus is unlikely to have a single cause, it is a network phenomenon involving multiple brain structures, neurotransmitters, and receptor types, and it is often associated by hearing impairment. Therefore, it is unlikely that we will find a single treatment to address the problem. It is now recognized that each tinnitus patient incorporates a unique medical, psychological, and social experience. Numerous recent studies recommend individualized management programs that use multimodal strategies designed to satisfy the needs of every patient. These studies have suggested that a tinnitus management team, consisting of an otolaryngologist, audiologist, neurologist, psychologist, and sleep or pain specialists, is crucial for successful tinnitus treatment.

Comprehensive tinnitus treatment includes assessment of hypertension, blood lipids, thyroid function and allergies. It also involves patient education on factors that aggravate tinnitus, like stress, caffeine, nicotine and aspirin.

2.3.2 Surgical treatments

Currently there is no surgical treatment option available. The destruction of the cochlea (cochleostomy) or the section of the auditory nerve that was performed decades ago, when the central genesis of the process was unknown, have been left behind. Fortunately, these treatments are no longer an option since they leave the patient totally deaf and increase the imbalance in the input of auditory information, which also worsened the tinnitus (Soleymani et al, 2011)

2.3.3 Pharmacological Therapies

Pharmacological therapies are available tools for doctors, where various drugs can be used (e.g, lidocaine i/v, benzodiazepines, baclofen, carbamazepine, Ginkgo Biloba, nimodipine, dihydroergotoxine, idebenone, among others). However, no drug has been shown to provide a replicable long-term reduction in tinnitus (Langguth et al., 2009).

2.3.4 Other Treatments

The knowledge that tinnitus is a product of the interaction between auditory dysfunction, cognitive changes in attention and emotional aspects such as anxiety and depression, has led to the development of various treatment paradigms such as teaching to differentiate between tinnitus and other sounds , improve the ability to discriminate, psychological treatments, relaxation techniques, cognitive-behavioral therapies, stress reduction, exercises, physiotherapy, acupuncture and electroacupuncture, among others (Han et al, 2009).

2.3.5 Tinnitus retraining therapy

One of the most widely used protocols is Tinnitus Retraining Therapy (TRT). The objective of TRT is to psychologically disconnect the patient from the tinnitus dependency, and concomitantly stimulate with soft and pleasant sounds to achieve its masking (Jastreboff, 1995; Von Wedel et al., 1997; McKinney et al., 1999; Kroener- Herwig et al., 2000).

2.3.6 Biofeedback

Biofeedback is a relaxation technique that teaches people to regulate certain autonomous bodily functions. The goal is to assist people to manage tinnitus-related distress by altering the patient's response to it. Many people have noticed an improvement of tinnitus after they can adapt their

response to it, although well-controlled studies haven't yet been completed (Güntensperger et al, 2017).

2.3.7 Other treatments

There are other treatments that are still in the research stage, such as electrical stimulation at the cochlear level or through deep implants in different nuclei of the auditory pathway up to the primary auditory cortex. The potential benefit of transcranial magnetic stimulation (De Ridder, et al., 2007; Kleinjung et al., 2007) and bimodal neuromodulation (Conlon et al., 2020) have also been studied.

2.3.8 Tinnitus and depression

Many tinnitus patients show signs of depression related to altered serotonin levels. Serotonin and GABA receptors are found throughout the sensory system and neurotransmitter abnormalities can play a role in some patients. Attempts to alleviate symptoms have included antidepressants (e.g., amitriptyline), anxiolytics (e.g., diazepam), anticonvulsants (e.g., clonazepam), diuretics and antihistamines (e.g., dexchlorpheniramine maleate), all of which produced inconsistent and inconclusive results (Baldo et al, 2012).

2.3.9 Tinnitus and hearing loss

In all patients who present with tinnitus there is, to a greater or lesser degree, an affectation of the auditory system that can range from sub-clinical hearing loss to mild, moderate or severe hearing loss (Han et al, 2009). At the same time, not all patients with hearing loss have tinnitus. Many times, it is the slightest hearing loss with small interatrial asymmetries that most "confuse" the auditory system, triggering attempts to homogenize the flow of information that end up generating tinnitus.

When a patient has concomitant hearing loss with an indication for hearing aids, the improvement in the balance of the auditory input is often sufficient to reduce the intensity of the tinnitus (König et al., 2006). However, in many cases, especially in young subjects, although there may be an alteration in the audiogram with the presence of asymmetries, this is generally mild and/or moderate and does not justify the indication of a hearing aid. The same occurs in cases in which the hearing alteration is sub-clinical, not being detectable in the conventional audiogram and being able -however- to identify alterations in the Otoacoustic Emissions (especially in the Transient ones, TEOAE) and/or in High Frequency Audiometry (HFA). It is in these cases that Acoustic Stimulation is considered as the first therapeutic tool against tinnitus.

2.4 Acoustic Stimulation for the Treatment of Tinnitus

Since most forms of severe tinnitus are caused by functional changes, it should be possible to reverse it with sound treatment, taking advantage of the plastic properties of the brain (Pedemonte et al, 2010).

Acoustic stimulation as an alternative in the treatment of tinnitus began to be used in the late 1970s (Vernon, 1977). The first devices used consisted of white noise generators that, through masking, produced an improvement in the quality of life of patients. This type of stimulation is called “Passive Function” since it does not involve changes in information processing that persist once the masking noise is not present. In other words, these types of treatments are effective only while the stimulating sound is present. Once the stimulating sound is removed, the tinnitus returns to the same characteristics it had before the stimulation began.

2.4.1 Active acoustic stimulation

In contrast to the above, in the early years of the 21st century, “Active Acoustic Stimulation” (AAS) protocols began to be used, seeking to generate persistent changes in auditory processing. These changes would be mediated by neuroplasticity phenomena that lead to a reorganization of the processing in the network, re-establishing the balance between inhibition and excitation in the neural groups involved. Different mechanisms have been postulated as being responsible for the changes observed in treatment with AAS, the most accepted being:

a) The increase in the preferentially inhibitory tone of the efferent system, which would produce a decrease in spontaneous discharge patterns (Hazell, 1987).

b) The prevention of spontaneous discharge synchronization phenomena in the auditory nerve (Eggermont, 1990; Moller 1995).

c)The prevention of the cortical reorganization that produces the input deficit in the primary auditory cortex, re-establishing the cortical representation of the affected frequencies and therefore their efferent projection on the neurons involved in the genesis of tinnitus (Eggermont and Komiya, 2000; Noreña and Eggermont, 2005).

d) The decrease of abnormal activity at the “edge” of the of cochlear damaged regions mediated by an increase of inhibitory tone of cholinergic synapses between the olivocochlear efferent system and the external hair cells (Roberts et al, 2013).

The evolution of research in the last two decades has shown that the best therapeutic results are obtained when sound stimulation takes into account some of the spectral and/or intensity characteristics of tinnitus (Schaette et al, 2010). To this end, different stimulation protocols have been developed that involve sounds or melodies with modifications in their component frequencies, white noise, pure tones with phase displacement, amplitude and modulated frequency, and different combinations of pure tones (Vermeire et al., 2007; Wilde et al., 2008; Wazen et al., 2011; Pantev et al., 2012; Reavis et al., 2012; Heijneman et al., 2012).

2.4.2 Acoustic stimulation during sleep

Since the 1980s, several tinnitus treatment methods have been developed that use sound stimulation as a therapeutic strategy. With the premise that there is a direct correlation between the spectral characteristics of the sound the patient hears and the frequency range in which there is a deficit in the provision of information that triggers tinnitus, a Uruguayan team of clinicians and researchers proposed two decades ago the idea of treating tinnitus with a sound that precisely replicates the features of the frequency range and intensity of each patient's tinnitus. The key was to provide the auditory system with very specific instructions so that the brain itself restores the balance between excitation and inhibition by providing the flow of lost acoustic information (Drexler et al, 2016).

The strategy was founded on understanding of the auditory system's great capacity for discriminating, which was established through hundreds of thousands of years of evolution in which hearing was honed as a warning mechanism against predators and as the primary channel of intra-species communication. The human ear has a great ability to discriminate in both dynamic range and frequency range. Taking into account these characteristics, the therapeutic strategy was created in order to communicate with the brain using precise acoustic patterns that targeted the relevant frequencies and intensities. To do this, the team created hardware and software that, when combined with a sophisticated sound synthesis technique, enabled them to create customized acoustic designs to reproduce each patient´s tinnitus. This customized sound was called Individualized Acoustic Receipt (IAR).

Through a process of re-education of the auditory system, mediated by neuroplasticity, the treatment seeks tinnitus compensation. The course of therapy lasts one year, during which time there are at least 19 in-person or online consultations with the various members of the clinician's team. In doing so, they support auditory stimulation and psycho-emotional habituation processes intended to mitigate tinnitus and lessen related symptoms (Drexler et a, 2016).

2.4.3 Why during sleep?

The protocol is used during nighttime sleep, in contrast to all acoustic stimulation protocols created in recent years, which apply sound stimulation during the day. Background information from earlier studies demonstrates that auditory processing continues during sleep and that there is a connection between learning, memory, and various stages of sleep (Velluti, 2008). The concept behind using the stimulation during the night time is based on the learning processes that takes place during sleep.

Administering the treatment while the patient sleeps also proved to be advantageous since in tinnitus patients, a vicious cycle between sleep and tinnitus is developed. Tinnitus tends to deteriorate with increased tinnitus intensity, which in turn tends to decrease sleep quality (Hurtuk et al, 2011). The moment of sleep consolidation is one of the hardest times for tinnitus patients since the reduction in ambient noise level that takes place at night makes tinnitus more noticeable.

Stimulation during sleep also has the advantage that it lessens the distress that tinnitus causes because patients do not need to focus on their tinnitus during the day and have it interfering with normal activities.

2.4.4 Acoustic stimulation during sleep and quality of life

Acoustic stimulation during sleep achieves a significant and sustained reduction of tinnitus intensity (Pedemonte et al., 2010). Previous studies demonstrated that there is a correlation between the reduction of intensity and the outcomes of psychological tests for the evaluation of tinnitus applied during the course of the treatment (Drexler et al.,2016).

3. Materials and methods

3.1 The SONUS system

The system comprises of two parts. The first part is an application called “back office” that runs on the clinician’s computer and the second part, called “SONUS player”, is another application that runs on the patient’s mobile phone. It also includes a pair of in-ear headphones, customised to the shape of the external ear canal of each patient.

The back office consists of a complex sound synthesiser which allows generation of individualised acoustic recipes (IAR). During the initial visit, the SONUS player is installed in the patient’s mobile phone and app navigation instructions are provided. In this visit, the sound engineer creates the IAR individualised to each patient, reproducing the volume and frequency content of the tinnitus.

The SONUS player allows communication with the back-office server and loads the individualised acoustic receipt of the patients. Every night, it gives the patients the possibility of calibrating the volume, but it restricts them from changing the frequency content of the acoustic receipt. The sound is delivered through the custom in-ear headphones.

One of the main features of the app is the intensity adjusting bar which allows the patients to adjust and set the intensity of the stimulation to slightly above the intensity of the tinnitus every night before going to sleep. The patient will undergo acoustic stimulation during sleep every night for the course of the treatment. The back office of the clinic receives the intensity that the patient has set, which allows the production of an evolution graphic to keep track of the response to the treatment (Figure 1). It also receives information about the hours of stimulation completed each night and the number of mistakes made in the use of the system in each stimulation session. In an event where the patient fails to comply with the treatment for one or more nights, the

intensity on the graph is left blank. In these cases, no value is added to the time series (i.e., missing entries are not filled with zeroes).

3.2 The inclusion criteria for the patients involved in this study

The inclusion criteria were: (1) patients from 18 to 70 years old with unilateral or bilateral subjective idiopathic tinnitus, (2) experiencing tinnitus for more than six months, and (3) a tinnitus handicap inventory (THI) score above 16. The exclusion criteria included patients that demonstrated: (1) objective or subjective secondary tinnitus, (2) hearing loss of 50 dB hearing threshold level (HTL) or worse in more than three frequencies of the audiogram, (3) patients that had undergone other treatments for tinnitus in the past year, (4) current use of hearing aids, (5) use of psychoactive drugs, (6) sleep disorders other than those provoked by the tinnitus itself. Patients with sleep disturbances such as apnoea, restless legs syndrome, narcolepsy, parasomnia and insomnia with aetiology other than tinnitus, were also excluded from the sample. In order to evaluate these criteria all patients were interviewed and examined by an otolaryngologist, an audiologist, and a psychologist. The laboratory profile consisted of imaging studies (MRI) and blood tests (blood lipids, thyroid hormones, glucose tests, urea, electrolytes and creatinine). Audiologic evaluation: Audiometric profiles were performed using impedanciometry, audiometry (exploring thresholds at 0.125, 0.25, 1, 2, 3, 4, 6, and 8 kHz), loudness discomfort levels (LDLs), speech audiometry and high frequency audiometry (8, 10, 12, 14 and 16 kHz). Two types of otoacoustic emissions (OAEs) were measured: distortion product otoacoustic emissions (DPOAEs) and transient evoked otoacoustic emissions (TEOAEs). DPOAEs were measured using three pairs of pure tones per octave in the range of 1–6 kHz. TEOAEs were studied with a broadband click in the range of 1–5 kHz.

3.3 Design of the individual acoustic receipt

During their initial visit to the clinic, the patients undergo consultation with a trained sound technician to create the designated acoustic receipt complementary to the acoustic features of tinnitus. The acoustic features of tinnitus may include the following patterns:

1. Pure tone- a sound with a sinusoidal waveform with a single frequency
2. White noise- a sound that contains all frequencies in equal measure
3. Broadband noise- a noise which has its energy distributed over a given section of audible range
4. Mixture of pure tone and broadband noise
5. Combinations of pure tones

In this last category, we have described a particular combination in which several pure tones in the form of a "cluster" (separated by low-amplitude frequency intervals) generate, from multiple phase cancellations, a type of tinnitus that we have called "cricket".

During an individualised acoustic receipt design, the patients are exposed to several pairs of sounds and asked at each step of the process if their tinnitus is similar to sound A or B. The responses from the options (sound A or B) are used to narrow and gradually discover the characteristics of the patient’s tinnitus.

The search algorithm and the associated software and hardware programs are updated and improved on a regular basis. This allows a trained sound technician to design and produce the acoustic receipt approximately within 20 minutes, making the procedure time effective. Details of the acoustic receipt sound design procedure have been filed with the US Patent Office (USPTO) in patent number US 9,282,917 B2 (Drexler et al. USPTO 2016).

3.4 Treatment with acoustic stimulation during sleep

The treatment lasts a year. It is divided into three phases: adaptation, continuous stimulation, and detachment. The clinical criteria for the division of the phases are described in the following.

3.4.1 The adaptation phase

The initial stage, called "adaptation stage" lasts between 1 and 2 months. In this stage, the patient learns to use the device, is trained in the calibration of intensities, and learns to sleep with it. Once the patient gets used to sleeping for at least five hours per night with the device and gets the ability to navigate the app and calibrate the volume every night, the next phase begins.

3.4.2 The continuous stimulation phase

The second stage is "continuous stimulation"; it lasts 3-4 months in which the patient wears the device every night. During this phase, tinnitus intensity tends to decrease until it reaches a lower plateau. Once the clinician notices no further decrease of intensity, he/she decides to progress the treatment onto the last phase.

3.4.3 The detachment phase

The third and final stage is called "detachment" and lasts between 5 and 7 months until the year of treatment is completed. Following the reduction in the intensity, the patient may be asked to reduce the number of days in a week for using the RAI treatment. This means, for instance, a patient may start using the treatment six days in a week for a few weeks. During the regular visits to the clinic, the consultant may ask to progressively reduce the treatment days at the end of the continuous stimulation phase. This is labelled as the end of continuous stimulation and start of the detachment phase. The detachment phase allows the patient to start detaching from the treatment. The evolution graphic is used to determine if the resulted reduction in the tinnitus intensity is maintained without acoustic stimulation every night. By the end of the detachment phase, the patient has a reduced tinnitus and does not require the acoustic receipt stimulation, marking the end of the treatment. At this stage, the days per week of stimulation are progressively reduced, seeking that at the end of the year the patient is no longer using the device on a regular basis. The evolution graphic is used to determine if the resulting reduction in the tinnitus intensity is maintained without acoustic stimulation every night.

3.5 Volume time series and data analysis

Each night, the patient adjusts the volume of the device playing the acoustic receipt to be slightly above the volume of the perceived tinnitus. This value is recorded in the mobile phone app and transmitted to the doctor's computer. After adjusting the volume, the patient goes to sleep with the acoustic stimulation being delivered via the app.

The clinicians had the impression that the patients that had more variability in the volume during the adaptation phase performed better at the end of the treatment, in the sense that they achieved more reduction of the tinnitus volume at the end of the continuous stimulation phase. To statistically test this observation, we performed a linear regression between the variability in the adaptation phase and the volume reduction achieved in 18 patients. The volume reduction was calculated as the volume of the acoustic receipt minus the median of the volume recorded by the patient in the last 10 days of the continuous stimulation phase. For each patient, the volume reduction was expressed as a proportion of the acoustic receipt volume. The linear regression was calculated using data from the ear that was more affected by the tinnitus in each patient. This was determined by the acuphenometry, which measures the tinnitus characteristics of a patient, and what the patient mentions in the clinical history. In the patients where the tinnitus had the same intensity in both ears, we used an average of both sides.

The variability during the adaptation phase was characterized using 9 different indexes, each one linearly regressed against the volume reduction using the data of the 18 patients (Table 1, 2 and 3). For each linear regression, we performed an F test to determine if the points followed an increasing trend, meaning that the more variability implied more volume reduction of the tinnitus. The null hypothesis was that the line was horizontal, i.e., the linear regression was not significantly different than a model done using the intercept alone. If $p < 0.05$, we rejected the null hypothesis, being able to claim that the fitted line was increasing. The variability indexes tested were the following: 1) standard deviation (SD), 2) standard error (SE), 3) range, 4) skew up from the median, 5) skew down from the median, 6 and 7) area of the histogram above and

below the median, 8) order of the autocorrelation function (d) in which the function reached a value < 0.5, and 9) area of an embedding created by plotting each variability value i against i+d, joining the first and last values to close a polygon. We evaluated the p value obtained in the F test for each of the 9 indexes tested (Table 3). As this operation involved 9 comparisons, we corrected the p values using false discovery rate (FDR) to obtain q values. A p value < 0.05 means that the risk of obtaining a false positive was less than 5%. A q value of less than 0.05 in the FDR test means that the risk of obtaining a false positive was less than 5% of the cases having p < 0.05. The significance level was set at p < 0.05.

4. Results

The bar chart in Figure 1 represents the volume that a patient measured every night before going to sleep. The horizontal red line at the top is the level of the IAR, which is the level of sound determined by the doctor during the initial visit to the clinic when they set up the device and treatment. The blue bars represent the intensity (in dB) set by the patient each night, received in the clinic's back office through the SONUS application. This information was used to produce an evolution graphic throughout the course of the treatment. In the days when the patient did not comply with the treatment, the space in the bar chart was left blank.

In each patient, the evolution was divided into 3 phases: 1) Adaptation phase, 2) Continuous stimulation phase, and 3) Detachment phase (Figure 1). The IAR was compared with the volume values at the end of the continuous stimulation phase. This patient shows a decrease from 50 dBs to around 30 dB, which may not seem like a dramatic difference at first sight. However, as the decibel scale is logarithmic, every 6 dBs means a reduction of the tinnitus volume by half. The 20 dB reduction achieved in this patient implies that the tinnitus volume was reduced to a 10% of its initial level.

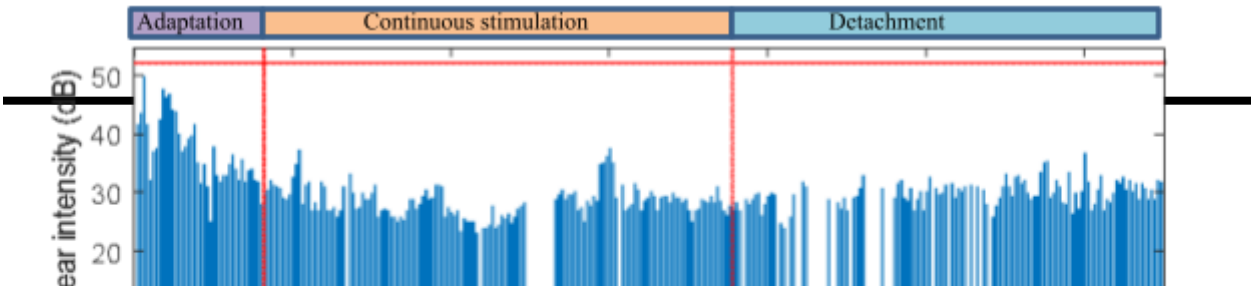

Figure 1. Bar chart showing the volumes measured by a patient every night throughout the course of the treatment. The y axis shows volumes in dB and the x axis the stimulation days. Treatment phases are indicated on the top of the figure. The days in which no stimulation was carried on are left as blank spaces.

The hypothesis that we want to test is that variability of the blue bars in the adaptation phase can predict how large will be the volume reduction. The volume can be different for each ear, so in each patient we used the ear having the loudest tinnitus or the mean of both ears if there was no difference. As the tinnitus volume (measured in dB) varies very considerably within patients, we normalized all entries (blue bars) to the volume of the IAR. In this way, we obtained a time series of volumes expressed as a proportion of the IAR of each patient (Figure 2-A). This operation allows the volume differences to be comparable in all patients. We confirmed previous results regarding the effectiveness of the treatment (Drexler et al., 2016) with a mean reduction of 17.67 % in the normalized tinnitus volume of the 18 patients recruited for the present study ($p = 4.55 \times 10\text{-}4$ in paired Wilcoxon test).

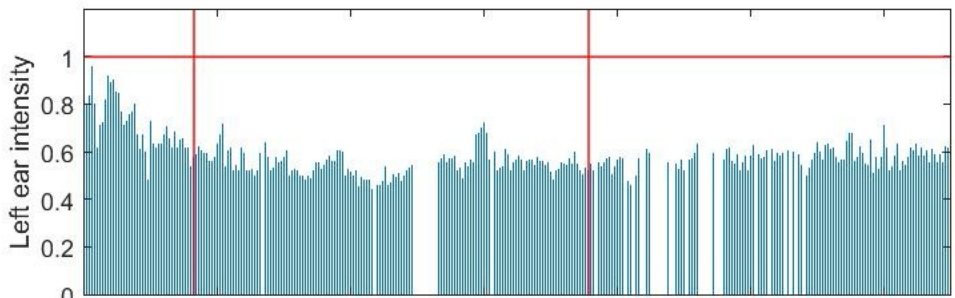

Figure 2. A) Bar chart showing the volumes measured by a patient every night throughout the course of the treatment. In this case the y axis shows volumes normalized to the IAR. B) Amplitude histogram of the variability during the adaptation phase. The median is indicated with a vertical red line.

Nine indexes from the variability of the normalized time series during the adaptation phase of each patient were calculated (Table 1). The last column shows the volume difference. The amplitude histogram of the adaptation phase variability is represented in Figure 2-B for the same patient depicted in Figures 1, 2-A and Table 1. Following this procedure for all patients we compiled a table with the data from the selected ear (or the mean of both ears) in all 18 patients (Table 2). We then performed a linear regression of each column of Table 2 (containing the different variability indexes) against the last column of the volume difference. The linear regression results are shown in Table 3, with the variability indexes in the rows. The columns of Table 3 show the correlation coefficient, the p-value (F test) and the adjusted R squared of each linear regression. Notice that the first four variability indexes have $p < 0.05$. This confirms the hypothesis that the larger the variability in the adaptation phase the more pronounced is the volume reduction of the tinnitus. The smallest p value was reached by the skew up of the variability (i.e., the distance from the median to the maximum of the distribution). This means that the variability in the direction of tinnitus volume increases is the best predictor of the treatment effectiveness. The measures that evaluate the variability in both senses (SD, SE and range) were also significant ($p < 0.05$) but to a lesser extent, as indicated by larger p values. The variability in the sense of volume decrease during the adaptation phase (skew down) was not significant, as was also the case for the areas of the histogram in the two sides of the median.

	SD	SE	range	skew_up	skew_down	area up	area down	autocorr_time	poly_area	Dif
right ear	0.101989098	0.015928021	0.633963277	0.358409651	0.275553626	0.48780488	0.51219512	2	0.009633533	0.576032155
left ear	0.109500999	0.017101183	0.476187822	0.273836406	0.202351417	0.51219512	0.48780488	3	0.035175954	0.422808611
mean	0.105745048	0.016514602	0.55507555	0.316123028	0.238952521	0.5	0.5	2.5	0.022404744	0.499420383

Table 1. An example of the table constructed for each patient showing the nine indexes used to characterize the variability and the difference of volume achieved (last column). In this patient (same patient as shown in Figure 1 and 2), the tinnitus volume was larger in the left ear.

The last two variability indexes, which inform about the autocorrelation properties of the adaptation phase time series, were also not significant. We wanted to assess if the autocorrelation properties of the time series are influential or if it is just the amplitude of the variability where the prognosis value can be found. The autocorrelation function decaying time estimation (d) and the area of the embedding created by plotting each value of the adaptation variability (order i) against the value at order i+d (joining the first and last values to close a polygon) had $p > 0.05$. In this context, we concluded that the indexes that measure the amplitude of the variability are the only ones that inform about the treatment outcome.

Patient	Ear	SD	SE	range	skew_up	skew_down	area up	area down	autocorr_tim	poly_area	Dif
patient1	left ear	0.1095	0.0171	0.4762	0.2738	0.2024	0.5122	0.4878	3	0.0352	0.4228
patient2	mean	0.0702	0.0078	0.5125	0.2164	0.2961	0.6159	0.3841	2.5	0.0306	0.1781
patient3	mean	0.0826	0.0118	0.3156	0.263	0.0526	0.4082	0.5918	3	0.0116	0.3025
patient4	right ear	0.1965	0.0248	0.7618	0.2516	0.5103	0.5873	0.4127	2	0.169	0.2563
patient5	right ear	0.0848	0.0109	0.546	0.2661	0.28	0.5738	0.4262	2	0.0533	0.1312
patient6	mean	0.1134	0.0128	0.4762	0.2748	0.2014	0.5063	0.4937	2	0.0535	0.3512
patient7	right ear	0.0973	0.016	0.371	0.1955	0.1755	0.5135	0.4865	2	0.029	0.3546
patient8	left ear	0.0401	0.0052	0.1939	0.1263	0.0676	0.6271	0.3729	2	0.0026	0.0655
patient9	right ear	0.1292	0.0202	0.6281	0.1611	0.4669	0.3902	0.6098	2	0.0077	0.1605
patient10	mean	0.1341	0.0207	0.5487	0.311	0.2377	0.4524	0.5476	2	0.1059	0.1983
patient11	mean	0.0349	0.0061	0.1577	0.044	0.1137	0.1212	0.8788	2	0.0028	-0.0652
patient12	mean	0.1295	0.0169	0.6938	0.3699	0.3238	0.8136	0.1864	4	0.122	0.1997
patient13	right ear	0.1165	0.0147	0.4288	0.0509	0.3779	0.5238	0.4762	2	0.0253	0.106
patient14	mean	0.0804	0.0114	0.2602	0.1557	0.1046	0.56	0.44	2	0.0174	0.128
patient15	left ear	0.0431	0.0067	0.1569	1.62E-04	0.1567	0.5476	0.4524	2	0.0049	2.62E-05
patient16	mean	0.0783	0.016	0.2827	0.0883	0.1944	0.5833	0.4167	2.5	0.0105	-0.0402
patient17	mean	0.061	0.0111	0.243	0.1181	0.1249	0.7167	0.2833	2	0.0139	0.1808
patient18	left ear	0.1546	0.0216	0.7205	0.168	0.5525	0.451	0.549	2	0.0482	0.2499

Table 2. Variability indexes and volume difference in the 18 patients. For each patient we used the ear with higher tinnitus volume or the mean of both ears in cases with no clear lateralization. The last column shows the volume difference.

Variability measure	r	p	AdjR^2	FDR
SD	0.535631	0.021965	0.242332	0.098843
SE	0.468617	0.049812	0.170827	0.112076
range	0.495992	0.036312	0.198884	0.108937
skew_up	0.694504	0.001383	0.449982	0.012443
skew_down	0.176161	0.484407	-0.02953	0.547989
area up	0.15167	0.547989	-0.03806	0.547989
area down	-0.15167	0.547989	-0.03806	0.547989
autocorr_time	0.246508	0.324089	0.002064	0.486133
poly_area	0.352822	0.150973	0.069764	0.271751

The variability of the adaptation phase in the direction of volume increase provides the best predictor of the tinnitus reduction. In Figure 3 we show a plot of this linear regression. The best line fitted by the minimum squares' method is represented with a green trace. The values of correlation coefficient (r), p-value in the F test and adjusted R square and printed in the figure. The F-test result confirms that the green line has a significant increasing trend, meaning that a

Table 3. Linear regression results for each variability index (rows), depicting correlation coefficient (first column) P value in the F test (second column), adjusted R squared (third column) and q value in the FDR correction for multiple comparisons (forth column).

larger skew up is associated with a larger volume reduction. The adjusted R square indicates that the linear regression explains only 45% of the variability, suggesting that there exist other factors involved in the volume reduction of the tinnitus. This is expected by the clinicians and may involve individual differences in the pursuit of the treatment among other factors that are extremely difficult to control in a prolonged medical treatment (see section Discussion). Despite the considerable dispersion of the crosses with respect to the green line in Figure 3, the F test indicates a significant increasing trend, which proved that the initial clinical impression of the medics was correct. As nine F tests were performed, the p-values were further corrected using FDR to ensure the absence false positives, even if we accept certain risk of false negatives. The FDR corrected p-value (known as the q value) was < 0.05 for the skew up (forth column of Table 3), confirming its reliability as a predictor of the treatment outcome. We checked that the histograms of the variability during the adaptation phase (e.g., Figure 2-B) were not symmetric in most cases, failing a test for Gaussian distribution. For this reason, we abstained from fitting any curve to the histograms and adopted variability indexes derived from the actual distributions.

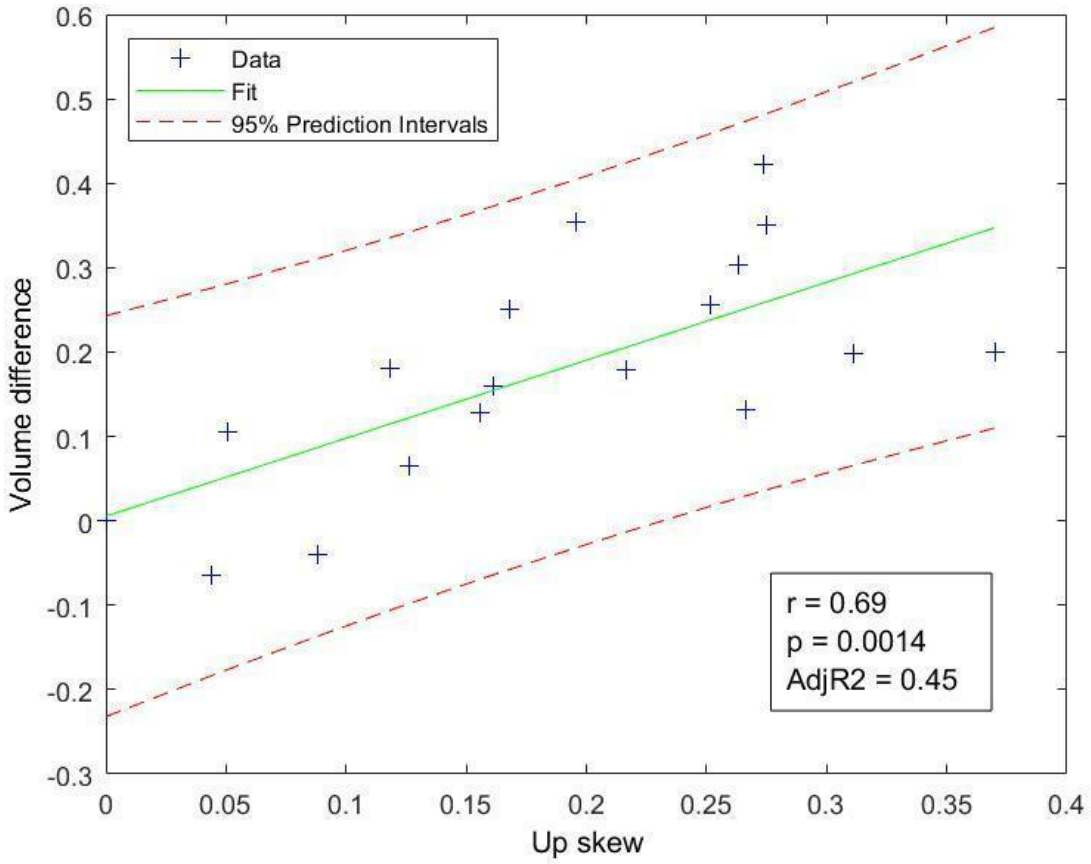

Figure 3. Linear regression of the variability histogram skew up during the adaption phase with the volume difference. The best line fitted to the data is represented with green trace. It has a significant increasing trend as shown by the p value of the F test (printed in the panel).

The transient volume increases during the adaptation phase (reflected by the skew up) are an indication of days when the treatment outcome appeared to go strongly in backwards direction (i.e., the tinnitus got temporarily worst). In the next section, we provide an explanation for this result, that may look paradoxical without being considered on the light of the complete time series of the acoustic stimulation treatment.

5. Discussion

The ability to modify the weight of synaptic contacts in response to changes in the environment or therapeutic stimulation is known as neuroplasticity (Markham and Greenough, 2004). In the present study, auditory acoustic stimulation is used as an attempt to reduce the volume of the patient's tinnitus. This is achieved through customised acoustic stimulation, which induces changes in the synaptic connections of the auditory pathway. The underlying microscopic and macroscopic mechanisms of neuroplasticity play a significant role in the long-term reorganization of auditory neural networks (Tonti et al, 2021).

5.1 Importance of auditory plasticity

Neuroplasticity is vital in the growth and development of an organism's sensory system. Although it takes place mostly during developmental stage (childhood), in adults the neurons of the brain can regenerate and recover after an injury (Markham and Greenough, 2004).

Alterations of auditory inputs can modify the auditory pathways, leading to an imbalance of the excitatory and inhibitory mechanisms, which may result in abnormal perception of sounds. Since most forms of severe tinnitus are caused by functional changes, it should be possible to reverse them with sound stimulation treatment, taking advantage of the plastic properties of the brain (Pedemonte et al, 2010). In evidence, research has shown that patients who received hearing aids or cochlear implants for their hearing loss also reported improvement in their tinnitus (Kleinjung et al, 2009; Servais et al, 2017). Since the late 80s, multiple acoustic stimulation protocols have been developed as an attempt to reverse the neuroplastic changes that underlie tinnitus (Wilde et al, 2008). Indeed, these treatments intended to reverse those pathological neuroplasticity changes by inducing further neuroplastic reorganization of the neural networks.

There is a broad range of evidence pointing to the fact that acoustic stimulation can induce remodelling of the neural network at every level of the auditory pathway (Rojas et al, 2018).

Tonotopic maps are modifiable, particularly in the auditory cortex, by using the appropriate acoustic stimulation (Langers and Dijk, 2012).

5.2 The role of underlying auditory plasticity in the intensity fluctuations of tinnitus volume during the adaptation phase of the treatment

Chronic tinnitus has been compared with focal epileptic seizures, in the sense that it is the result of spontaneous firing due to uncontrolled neural activity that escapes the inhibitory mechanisms of the brain (Adjamian et al, 2014). This may be the footprint of long-term abnormal flux of information in the auditory pathways, creating changes that are difficult to revert. The ability to overcome these changes by reorganizing the neural network is related to the neuroplastic capabilities of each person. These capabilities may be depended on several factors, for instance, age, learning skills and associated pathological conditions related to neurodegenerative diseases and mental health. In this context, the importance of social abilities has been highlighted by psychologists as a factor that may affect the neuroplastic capability of a person. For example, positive attitude, resilience and having a supportive social environment are widely recognised as influential factors in the outcome of any learning processes (Davidson and McEwen, 2012).

The hypothesis that we attempted to prove in this study is that the variability of the tinnitus volume during the adaptation phase has a prognosis value on the volume reduction achieved at the end of the continuous stimulation phase. Our tenant is that these fluctuations are related to the neuroplastic capacity of each patient's brain. This may bear similarity to a phenomenon known as residual inhibition (RI), which refers to a transient tinnitus loudness suppression followed by a remarkable rebound after exposure to an acoustic stimulus (Feldman, 1971). RI is less present in patients with chronic tinnitus or hearing impairment, suggesting that neuroplastic mechanisms are likely to be involved (Hu et al, 2021). In other words, the patients with less variability in the adaptation phase may have less neuroplastic capacity because the tinnitus has reached a chronic state or their global learning skills are compromised. On the contrary, the

patients with a higher variability may have retained a larger neuroplastic capacity and for this reason they respond better to the acoustic stimulation treatment.

After performing a linear regression between the variability during the adaption phase and the achieved volume decrease, we were able to demonstrate that the line of best fit showed a significant increasing trend (Figure 3, Table 3). Therefore, we can reject the null hypothesis. The adjusted R-squared value of 0.45 (Figure 3) means that there exist other factors that are influential in the volume reduction, as expected in any clinical intervention. Although the model shows a significant increasing trend (Figure 3), it explains less than half of the observed variability. As other factors that influences the treatment outcome, we can mention, age, onset time of the tinnitus, stress related to the day-by-day challenges of modern life, compliance with the treatment, audiologic profile and associated pathologies.

From the different indexes extracted from the variability in the adaptation phase, the model that shows a greater increasing trend (in terms of the P-value in the F-test) was the one performed using the skew-up. Some of the indexes that evaluate the variability in both senses (both increases and decreases in volume), also showed a significant increasing trend in the linear regression, which was not the case for the skew-down, that accounts for decreases in the volume. The skew up reflects the days in which a volume rebound was observed. Our interpretation of this fact is that the skew up may be reflecting a rebound in volume that may be similar to the one observed in the RI phenomenon.

It may seem paradoxical that these drawbacks, that can have a devastating psychological impact on the patient's perception of the treatment evolution, can be associated with a better outcome at the end of the treatment. This fact needs to be explained to the patient beforehand and should be included in the consent document. A network of contention involving different health professionals with special training must be put in place.

6. Concluding statement

In this work, we have demonstrated that the variability during the adaptation phase has a prognosis value on the treatment outcome. Out of all the indexes compared, the skew up showed the most significant increasing trend and the better fitting of the points to the adjusted line. These conclusions are relevant to the way in which management and monitoring are managed by a multidisciplinary team of health professionals. They also provide relevant clues to the provision of counselling that must be put in place for a successful outcome. Further research is needed to establish the importance of other prognostic factors, i.e., an evaluation of residual inhibition, learning capacities, spectral characteristics of tinnitus, etc. This research area has a promising future to understand in which patients' acoustic stimulation during sleep should be applied and why some patients respond better than others.

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