Electromagnetic Correlates of Injury-Induced Auditory Cortical Plasticity: Implications for the Development and Maintenance of Subjective Tinnitus

Dissertation
zur Erlangung des akademischen Grades
des Doktors der Naturwissenschaften
an der Universität Konstanz (Fachbereich Psychologie)
vorgelegt von Nathan Weisz
Mai 2004

Tag der mündlichen Prüfung: 28.07.2004
Referent: Prof. Dr. Thomas Elbert
Referent: Prof. Dr. Harald Schupp

Danksagung

Drei Jahre ist es nunmehr her, als ich mich auf dem Weg von Eichstätt ins hirnforschungslastige Konstanz machte um bei einem mir persönlich noch unbekannten Professor über ein mir völlig unvertrautes Thema mit mir teilweise absolut fremden Methoden (was in aller Welt ist MEG?) zu promovieren. Für die Chance diesen Weg zu beschreiten und seine Unterstützung möchte ich mich herzlich bei Thomas Elbert bedanken. Weiteren Dank gilt natürlich Brigitte Rockstroh, die mit ihrem Organisationstalent und Engagement einen Großteil der Rahmenbedingungen schafft, in der Forschung in Konstanz Spaß macht. Dies gilt ebenso für das technische Talent von Christian Wienbruch, ohne den das MEG-Labor wohl nicht funktionstüchtig wäre. Diskussionen zu Tinnitus und Aufklärungen bezüglich MEG haben mich viel weitergebracht. In diesem Rahmen muss auch Patrick Berg erwähnt werden, der immer einen nützlichen Tipp in Fragen Datenanalyse parat hatte. Inhaltlich und methodisch habe ich auch sehr viel von Andreas Keil lernen können, der – trotz gehetzten Terminplans und ohne eigentliche betreuerische Verpflichtung mir gegenüber – sich viel Zeit mit mir genommen hat. Ich weiß es sehr zu schätzen. Behalte diese tollen Tugenden bei, sie werden Dich sehr weit bringen! Konstanz wäre nicht einmal halb so schön gewesen, wenn die Chemie mit denjenigen nicht gestimmt hätte mit denen ich de facto die meiste Zeit in den letzten drei Jahren verbracht habe: d.h. alle meine Ko-Doktoranden. Allen voran die Pausen in der Kaffeküche und die Postkolloquia mit einer ausgewogenen Mischung aus inhaltlich guten Diskussionen und nicht selten auch einfach wohltuendem Schwachsinn bleiben mir positiv in Erinnerung. Danke auch an die Studenten Thomas Hartmann (Hilfskraft der ersten Stunde) und Sandra Voss, die bei Durchführung und Auswertung der hier vorgestellten Studien mitgeholfen haben. Viel Glück Euch beiden bei Praktikum bzw. beim Berufseinstieg!

Obwohl möglicherweise eine Selbstverständlichkeit möchte ich nicht zuletzt meinen Eltern danken, deren kontinuierlicher Rat und Unterstützung nicht mit Worten eingeschätzt werden kann. Ich widme ihnen daher diese Arbeit. Tanja: danke für die letzten 6 Jahre mit Dir. Nu-dja, der Rückblick auf diese Zeit entlockt mir ein Lächeln.

Konstanz, Mai 2004

Nathan Weisz

Table of Contents

ZUSAMMENFASSUNG 1

SUMMARY 3

1. INTRODUCTION 5

1.1. HEARING IMPAIRMENT AND PERIPHERAL APPROACHES 7
1.2. CENTRAL MODELS 9
1.2.1. Injury- and use-induced plasticity 10
1.2.2. Reorganization of the auditory cortex after peripheral damage 13
1.2.3. Reorganization of the auditory cortex and its potential relation to tinnitus 15
1.2.4. Behavioural relevance 21
1.3. TEMPORARY SUMMARY 23

2. GENERAL METHODS 25

2.1. MEG / EEG RECORDING APPARATUS 25
2.2. ARTEFACT CORRECTION 25
2.3. SOURCE ANALYSIS 26
2.3.1. Multiple equivalent current dipole fitting approach 26
2.3.2. Minimum Norm Estimate 28
2.4. HEARING THRESHOLDS AND TEST DETERMINATION OF FREQUENCIES 29
2.5. ASSESSMENT OF TINNITUS RELATED DISTRESS 30

3. EXPERIMENTS 31

3.1. STUDY 1: SPONTANEOUS ACTIVITY 31
3.1.1. Aim 31
3.1.2. Methods 32
3.1.3 Results 34
3.1.4. Discussion 38
3.2. STUDY 2: AUDITORY MISMATCH RESPONSE 40
3.2.1. Aim 40
3.2.2 Methods 40
3.2.3 Results 42
3.2.4. Discussion 47
3.3. STUDY 3: INDICATORS OF ALTERED TONOTPIC REPRESENTATION 49
3.3.1. Aim 49
3.3.2. Methods 50
3.3.3. Results 52
3.3.4. Discussion 59
3.4. SIDE-EFFECT: STEEPNESS OF HEARING LOSS AS AUDIOMETRIC CORRELATE OF DISTRESS 63
3.5. SUMMARY OF MAIN FINDINGS 65

4. GENERAL DISCUSSION.....66

4.1. TINNITUS A CONSEQUENCE OF MAP REORGANIZATION? 66
4.2. TINNITUS A CONSEQUENCE OF ALTERED SPONTANEOUS ACTIVITY? 67
4.3. PERIPHERY AND PLASTICITY 68
4.4. A TEMPORARY WORKING MODEL 71

5. PERSPECTIVES 77

REFERENCES 79

APPENDIX A 92

APPENDIX B.....114

Zusammenfassung

Die Wahrnehmung einfacher akustischer Reize (z.B. Pfeifen / Ton, Rauschen, Summen etc.) in Abwesenheit einer identifizierbaren physikalischen Quelle (äußere und körpereigene) bezeichnet man als subjektiven Tinnitus. Das häufige gemeinsame Auftreten mit einem messbaren Hörverlust veranlasste in den 1970-80'er zur Annahme, dass das 'Tinnitus-Signal' in der Peripherie des auditorischen Systems (Haarzellen, Hörnerv) erzeugt wird. Seit den 1990'ern setzte sich immer mehr die Ansicht durch, dass *Tinnitus im zentralen Nervensystem durch einen verminderten sensorischen Einstrom (wie z.B. bei Innenohrschädigung) in die relevanten Repräsentationsareale ensteht*. Dem Kortex kommt selbstverständlich als Ebene der bewussten Wahrnehmung dabei eine besondere Rolle zu. Welche Prozesse allerdings hauptverantwortlich für die Enstehung und Aufrechterhaltung von Tinnitus sind ist umstritten. Vereinfachend gesehen existieren zwei Forschungslinien: Die erste (*map reorganization*) ist stark geleitet durch Ergebnisse aus der Phantomschmerzforschung bei Gliedmaßenamputation, die zeigen, dass die deafferentierte Region ihr Antwortverhalten in Richtung intakter Nachbarbereiche verschiebt. Das Ausmaß dieser Reorganisation ist stark korreliert mit der Ausprägung des Schmerzes. Tinnitus wird oft als ein Phantomschmerz des Ohres gesehen, und eine analoge *map reorganization* wird dann als Ursache angenommen. Die zweite Forschungslinie – mit stark tierphysiologischen Wurzeln – vermutet den zugrundeliegenden Mechanismus in veränderten *Spontanaktivitätsparametern*. Vor dem Hintergrund der eben beschriebenen Mechanismen war das Ziel der Studien, die dieser Arbeit zugrundeliegen, die Identifizierung kortikaler Korrelate von subjektiven Indikatoren des Tinnitus mittels EEG und MEG. Alle Studien wurden mit Tinnituspatienten durchgeführt, die einen Hochtonhörverlust hatten, da eine Hörschädigung theoretisch als Basis für Tinnitusentstehung gesehen wurde. Es wurde angenommen, dass sich *map reorganization* speziell in einer erhöhten neuronalen Antwort bei audiometrisch normalen Randfrequenzen (lesion-edge, LE) widerspiegelt. In keiner Studie (insgesamt 2) konnte dies so bestätigt werden. Dennoch gab es einige LE-spezifische Effekte, der Wichtigste ein komplexes Mismatchmuster (Studie 2), das allerdings kaum vereinbar ist mit einfachen *map reorganization* Annahmen. Zudem zeigte sich diesbezüglich, dass hohe Tinnitusbelastung mit einem normaleren Mismatchmuster assoziiert war. Der deutlichste Hinweis für eine *map reorganization* liegt in einer

rechtshemisphärisch größeren Distanz (Studie 3) zwischen der Quellenlokalisation für den LE-Reiz und einem eine Oktave darunter liegenden Kontrollreiz: Allerdings korrelierte diese Variable nicht mit subjektiver Tinnitusbelastung. Ein weiterer Effekt zu *map reorganization* waren teilweise sehr deutliche Hemisphärenunterschiede zu normalhörenden Kontrollpersonen (Studie 3). Als konsistentes Korrelat für Tinnitusbelastung erwies sich die Quellenlokalisation in der posterior-anterior Richtung (Studie 2 und 3), wobei eine hohe Belastung mit weiter vorne liegenden Quellen einherging. Bezüglich Spontanaktivität (Studie 1) wurde eine Erhöhung v.a. im Delta-Bereich speziell in temporalen Arealen erwartet. Grund dafür ist, dass langsame oszillatorische Hirnaktivität in der Literatur als eine Begleiterscheinung deafferentierter neuronaler Netzwerke diskutiert wird. Tatsächlich konnten Veränderungen in der Spontanaktivität gefunden werden, die in diese Idee stützen. Ein wesentlich stärkerer Effekt war aber eine Alpha-Reduktion in temporalen Gebieten. Mit der Ausprägung der Tinnitusbelastung assoziiert, zeigten v.a. rechtstemporale und linksfrontale Regionen hohe Korrelationen. Als ein Nebeneffekt der Studien konnte ein negativer nonlinearer Zusammenhang zwischen Tinnitusbelastung und Steilheit des Hörverlusts gefunden werden: i.e. hohe Belastung ging häufig mit flacheren Hörverlusten einher. Letzterer Befund deutet darauf hin, dass die Beschaffenheit der Hörschädigung einen zentralnervösen Einfluss hat, der mit Tinnitus assoziiert ist. Zudem unterstreicht dies insgesamt die Bedeutung der Hörschädigung. Zusammenfassend kann man sagen, dass zahlreiche Auffälligkeiten in den EEG- und MEG-Daten bei Tinnitusleidenden gefunden werden konnten. Die Daten deuten darauf hin, dass *map reorganization* als einzige Erklärung nicht ausreichend ist. Vielmehr könnten einige Mechanismen für die Entstehung und Aufrechterhaltung von Tinnitus (und somit Therapieresistenz) in einem netzwerkartigen Wirken von veränderten peripheren Mechanismen (zu beobachten nach Hörschädigung) sowie emotionalen- und Aufmerksamkeitsprozessen auf auditorische Repräsentationsareale liegen. Elemente eines Arbeitsmodells, das diese Mechanismen sowie ihren Bezug zu den vorliegenden Ergebnissen berücksichtigt, werden zum Schluss diskutiert.

Summary

The term subjective tinnitus refers to the perception of simple sounds (e.g., tonal, noiselike, etc.) in the absence of an identifiable physical source. It is frequently accompanied by a measurable hearing loss, which lead to the assumption in the 1970-1980's that the 'tinnitus-signal' is generated in the periphery of the auditory system (hair-cell, auditory nerve). Since the 1990's, the notion prevails that *tinnitus is generated in the central nervous system via a reduced sensory input (e.g., following damage to the inner-ear) into relevant representational areas.* Naturally in this framework, the cortex – as the level of conscious perception – takes a special role. However which processes are responsible for the generation and maintenance of tinnitus is a matter of discussion. Stated in a simplified way, two research approaches can be distinguished. The first (*map reorganization*) is strongly guided by results from research on phantom limb pain following amputation, which could show that deafferented regions shift their response properties in the direction of intact neighbouring regions. The magnitude of reorganization is strongly correlated with the degree of pain. Tinnitus is frequently regarded as an auditory analogue to phantom limb pain, and an analogous *map reorganization* is assumed as the cause. The second research approach – with strong animal physiological roots – reckons the underlying mechanism to lie in altered parameters of spontaneous activity. Taking into account the background of the just described mechanisms, the aim of the present studies, that form the basis of this work, is the identification of cortical correlates of subjective indicators of tinnitus using EEG and MEG. All studies were conducted with tinnitus patients, who had high-frequency hearing loss, as a hearing damage is theoretically regarded as basis for development of tinnitus. It was hypothesized that *map reorganization* should be specifically reflected in an enhanced neuronal response for audiometrically normal edge-frequencies (lesion-edge, LE). This could not be confirmed in any of the studies (overall 2). Yet there were some LE-specific effects, the most important a complex mismatch pattern, which however is hardly compatible with simple assumptions of *map reorganization*. Moreover it appeared that strong tinnitus distress was associated with a more normal mismatch pattern. The strongest indication for *map reorganization* was a right –hemispheric enlarged distance (study 3) between the source location for the LE stimulus and a control stimulus an octave below: However this variable did not correlate with subjective distress. Another effect

concerning map reorganization were the partly pronounced hemispheric differences to normal hearing controls (study 3). Source location on the posterior-anterior axis turned out to be a consistent correlate for tinnitus distress (study 2 and 3), with strong distress being associated with more anterior sources. Regarding spontaneous activity (study 1) an enhancement was expected especially in the delta-region particularly in temporal areas. The reason for this is that slow oscillatory brain activity is discussed as a concomitant of deafferented neural networks. Indeed alterations of spontaneous activity could be observed that support this idea. A substantially stronger effect however was an alpha-reduction in temporal areas. Associated with the degree of tinnitus distress, particularly right-temporal and left-frontal showed strong correlations. A side-effect of the studies was a negative nonlinear correlation between tinnitus distress and steepness of the hearing loss: i.e., strong distress was frequently accompanied by more flat hearing losses. The latter finding indicates, that properties of the hearing damage has an central nervous influence, that is associated with tinnitus. It furthermore underlines the importance of the hearing damage. Overall one can state that several abnormalilities could be found in the EEG and MEG data of tinnitus sufferers. The data implicates that *map reorganization* is not sufficient as a single explanation. Rather some mechanisms for the generation and maintenance (and therefore is resistance to therapy) of tinnitus could lie in a network-like influence of altered peripheral mechanisms (observable after hearing damage) as well as emotional and attentional processes on auditory representational areas. Elements of a working model, that considers these mechanisms and their relationship to the present results are discussed at the end.

1. Introduction[1]

Subjective tinnitus (subsequently only termed *tinnitus*), i.e. the perception of a (usually high-frequency) sound (-s; e.g. pure tone, ringing, hissing etc.) in the absence of an objective physical source, is a phenomenon which has puzzled physicians for several thousand years (Feldmann, 1992). Even though explanatory attempts have changed considerably over the course of time, tinnitus has not lost much of its mysterious qualities. Up to now, there is no generally accepted and scientifically sound view how these phantom sounds come about, and – to the frustration of especially the ones affected – also no cure. That the latter aspect poses a problem which becomes clear after considering the high number of people affected: according to Pilgramm and colleagues (Pilgramm, Rychlik, Lebisch, Siedentop, Goebel, & Kirchhoff, 1999) approximately three million people (above the age of 10 years) in Germany report of suffering from tinnitus (point-prevalence), of which again the great majority (2.7 million) have had the symptoms for over a month. An exact quantification of prevalence is however difficult due to the different methods employed (e.g. Pilgramm et al. used telephone interviews, whereas Axelsson and Ringdahl (1989) sent questionnaires by mail). Also prevalence is strongly age dependent: frequency of tinnitus shows a gradual rise with increasing age, the age-group between 60-69 years being the most affected group (see Figure 1-1). Men older than 45 years are considerably more affected than their female peers (Lockwood, Salvi, & Burkard, 2002). Roughly half (1.5 million in Germany) of the tinnitus population are estimated to show mildly to strongly decompensated symptoms, e.g. sleep disturbance, concentration difficulties and psychiatric distress. A minority of tinnitus sufferers (0.5 %) are so severely affected, that they are unable to function properly in everyday life. Despite of its potentially distressing properties, tinnitus is not regarded as illness, but rather as a symptom that follows a damage to the auditory system: e.g. more than half of the patients experience an *audiometrically* testable hearing loss on the identical side as their tinnitus. The figures are similar for different industrialized nations (Meikle, 1997). Due to the growing exposure to noise in these countries, the incidence of otological disturbances and in particular tinnitus can be expected increase.

---

[1] Parts of chapter 1 and 4 are taken from a manuscript of a review article which is currently in preparation.

Recent animal and human neurophysiological studies have opened up a new path to the investigation of tinnitus, which might – in the long run – help to diminish the discrepancy between need and actual posession of knowledge. New treatment approaches could be the reward for these endeavors. The main goal of the work presented here is to identify neurophysiological correlates of tinnitus derived from ideas as to how the auditory cortex reorganizes following an impairment to the hearing system.

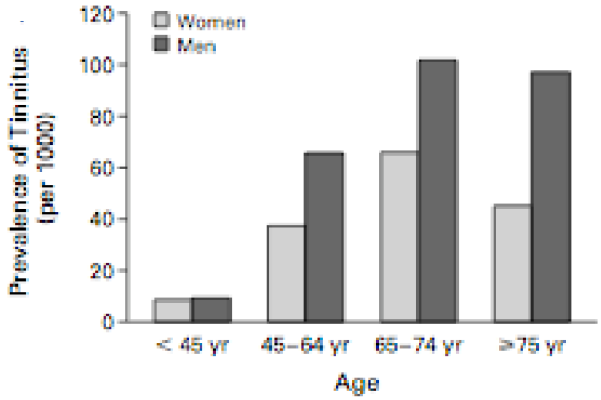

Figure 1-1: Prevalence of tinnitus shows an increase with age and men being more affected than women (taken from Lockwood et al., 2002).

The present chapter of this thesis attempts to give an overview of the main contributions of neuroscience to the understanding of tinnitus so far. Obviously, this has to remain highly selective and does not claim completeness. It will begin with an outline with what is considered to be the eliciting event: a damage to the peripheral auditory system (section 1.1.). Damage to auditory receptors is known to trigger changes to neural networks in the respective representational areas: this is as far as the general consensus goes. However, there is no general agreement about which changes might be involved in the generation and maintenance of tinnitus. A brief outline of results from research on *injury-induced* neuroplasticity will thus be offered in section 1.2., Here a parallel will be drawn to phantom limb pain following amputation, which is frequently thought to be a somatosensory analogue of tinnitus (Mühlnickel, Elbert, Taub, & Flor, 1998). These works form the knowledge fundament for the experimental part (chapter 3), which will be preceded by a description of the general methods (chapter 2). Based on the results presented in that part, an evaluation of the body of neuroscientific ideas surrounding tinnitus is given in the discussion (chapter 4). In the discussion I will also expound on own ideas that could contribute to the

large body of notions surrounding tinnitus. At the end I will attempt to point out potential future directions and developments in tinnitus research from a psychophysiological point of view.

1.1. Hearing impairment and peripheral approaches

The notion that the origin of tinnitus is intimately related to a hearing impairment stems from the observation that the majority of tinnitus sufferers have an audiometrically detectable hearing loss (Cahani, Paul, & Shahar, 1984). This situation usually follows a damage to hearing receptors of the inner ear (sensorineural hearing loss), in which outer hair cells (OHC) are more vulnerable than inner hair cells (IHC), and receptors close to the base (transducing high frequencies) more vulnerable than those close to the apex (transducing low frequencies; see e.g. Moore, 1995). Although a minority of cases, some tinnitus sufferers do not exhibit an audiometrically measurable hearing loss. This however, does *not* disprove an absence of a receptor damage as recently demonstrated by Shiomi, Tsuji, Naito, Fujiki and Yamamoto (1997). These authors were able to show significantly reduced distortion product otoacoustic emissions for a wide frequency range in 93 % of investigated ears (n = 15; 9 subjects) of tinnitus sufferers withouth hearing loss as compared to a control group without tinnitus sensation. This indicates a circumscribed damage of outer hair-cell function and reduction of frequency-selectivity. Normal thresholds in an audiogram (i.e., approximately up to 25 dB HL), may thus simply be the consequence of 'off-frequency' listening (Moore & Alcantara, 2001), i.e. of detecting the presented signal with neighbouring undamaged hair cells. A further reason why tinnitus is seen as connected to hearing impairment by many researchers, is because the pitch of the sensation falls into the range of the hearing loss in most cases (Norena, Micheyl, Chery-Croze, & Collet, 2002). Matching of tinnitus pitch – usually done as adjustment of a pure tone to match the perceived pitch – is known to be notoriously unreliable (Burns, 1984; Penner, 1983). In an interesting approach to elucidate the cause of this variability Norena et al. (2002) assessed the 'internal tinnitus spectra' of 10 participants, by letting them judge the contribution of different frequencies to their tinnitus sensation. The results were strongly related to the course of the audiogram of each individual, i.e. frequency components in the hearing loss range contributed most. The authors interpreted this as evidence that most tinnitus sounds consist of a

broadband spectrum, not excluding the possibility of a predominant pitch. However, the rationale of this and other studies investigating tinnitus pitch miss to consider a crucial point: a damage to receptors of the inner ear leads to altered perceptual consequences that are not limited to threshold (e.g., loudness perception, sound localization, temporal resolution; see Moore, 1995). One of the aspects of severely impaired hearing is frequency selectivity in the damaged area, which can be demonstrated in humans e.g. with psychoacoustical tuning curves (PTC; see e.g. Moore & Alcantara, 2001). PTCs are widened in hearing impaired persons, which has also been shown for tinnitus (Cazals & Dauman, 1990; Dauman & Cazals, 1989). There are thus reasons to assume that variability in matching seen across sessions (Burns, 1984; Penner, 1983) or across frequencies within one session (Norena et al., 2002) may simply reflect impaired frequency selectivity. **At least it has to be remembered that with a high probability pitch perception in tinnitus subjects does not function the same way as in normal hearing subjects**. Later in this thesis it will be argued that alterations of frequency selectivity (i.e., broader tuning and off-frequency listening) due to receptor damage may contribute to reorganization of auditory representational maps.

Despite the arguable success and usefulness of attempts to 'objectify' tinnitus by letting subjects match various psychoacoustical features, the merit of such studies lies in the demonstration that **tinnitus and hearing impairment are coinciding and probably somehow related phenomena**. Due to this coincidence, some authors – usually from the 80's to the early 90's – proposed models of tinnitus in which a signal, later interpreted by the brain as tinnitus sound, is generated in the peripheral auditory system (inner ear and hearing nerve) itself. The general notion was that following a deafferentation, hair cells or fibers of the auditory nerve fall into a *hyperexcitible* state. Correlated firing of neighbouring auditory nerve fibers due to abnormal cellular Ca^{2+} or K^{+} concentrations was proposed by Eggermont (1990). Synchronous activity in nerve fibers due to ephatic interaction following damage to the myelin sheath has also been named by Møller (1984) as the mechanism underlying tinnitus. Another argument serving as evidence for a peripheral generation of the tinnitus sound is that salicylate, which induces tinnitus in humans, leads to an increased spontaneous activity in the auditory nerve (Evans, Wilson, & Borerwe, 1981). Generally, formulated in an exaggerated manner, there is not a known dysfunction in the

peripheral auditory system which has not beed suggested to be relevant for tinnitus. An extensive overview of possible peripheral mechanisms is given by Jastreboff (1990).

Today, a broad consensus exists that the signal underlying tinnitus sensation is not caused in the auditory periphery. The main reason for this is that there is no strong evidence that spontaneous activity of hearing nerve fibers is enhanced in animals exposed to acoustic trauma (Muller, Smolders, Ding-Pfennigdorff, & Klinke, 1997; Salvi, Hamernik, & Henderson, 1983) or salicylate (Muller, Klinke, Arnold, & Oestreicher, 2003; except for very high doses, see Evans et al., 1981): either no changes or even a *reduction* of spontaneous activity are observed. Thus, a paradigm shift took place propagating a central generation of tinnitus (of course also motivated by the increasingly brain-interested *Zeitgeist*; see next section). Certainly, a pragmatic aspect for the failure of purely peripheral models is that all the research did not manage to breed a therapeutic approach offering a cure for this annoying condition. Even transections of the auditory nerve do not lead to a significant reduction or removal of the tinnitus sound in most cases, and sometimes the effects are counterproductive (Douek, 1987; House & Brackmann, 1981). The tight link between basic research on tinnitus and the high (clinical) expectations that these efforts should yield useful insights for therapeutic approaches may someday also judge over the fate of central models.

1.2. Central models

The consequence of the failure to explain tinnitus solely on the basis of peripheral mechanisms lead to the assumption that the tinnitus generating neuronal signal must arise within the central auditory system (Lockwood, Salvi, Coad, Towsley, Wack, & Murphy, 1998; Reyes, Salvi, Burkard, Coad, Wack, Galantowicz, & Lockwood, 2002). Most of the research in this direction has been conducted on animals and has been motivated by a generally increasing interest in the plasticity of sensory areas in the adult central nervous system, i.e. changes to the topographic manner in which the brain represents sensory information (tonotopic, somatotopic and retinotopic representation). Two important lines of research can be distinguished in this context (Elbert & Heim, 2001): injury- and use-induced plasticity. Both terms emphasize the brains ability to reorganize itself according to experience. Use-induced

plasticity refers to the neuroplastic reorganization to functional and morphologic changes of sensory and motor areas accompanying *learning*. With regard to tinnitus, this type of plasticity seems less important than insights gained from injury-induced plasticity, which deals with changes of sensorimotor areas following a damage to peripheral receptors. The following sections (section 1.2.1. and 1.2.2.) will give a brief overview of both types of plasticity, however with a focus on the injury-induced kind. Also, descriptions are largely restricted to the cortex due to spatial limitations and for the the pragmatic reason that most is known about it. In section 1.2.3. I will delineate how knowledge about injury-induced plasticity has been implicated in tinnitus. Additionally, some neuroscientific (especially neuroimaging) studies show that cortical reorganization does not occur without behavioural stimulation and / or context (section 1.2.4.). This seems particularly interesting knowing that tinnitus is sometimes accompanied by a considerable amount of distress, which could be an at least modulatory factor in cortical reorganization.

1.2.1. Injury- and use-induced plasticity

For a considerably long time, the majority of neuroscientists believed that the organization of sensory representational areas was stable beyond a certain stage of development (critical periods). This dogma however has been challenged especially in the last 20 years (Buonomano & Merzenich, 1998; Ramachandran, 1993) in which **evidence has accumulated that exemplify an astonishing capacity of the adult brain to reorganize itself**. Two major events elicit changes in the representational properties of neurons in sensory areas of the brain (Elbert & Heim, 2001): 1) behavioural training / learning that imposes a strong use of certain receptors of various modalities (*use-induced plasticity*) and 2) a damage to sensory receptors in the periphery (*injury-induced plasticity*). Even though the background and purpose are different for the two types of plasticity, the net effect on sensory neurons is similar.

An experimental approach to trigger off use-induced plasticity lies in conditioning (Weinberger, 1998). In the auditory modality, following aversive conditioning of certain frequencies (Edeline, Pham, & Weinberger, 1993; Edeline & Weinberger, 1993), auditory cortical neurons move their best frequency towards the conditioned stimulus. Similar effects have also been investigated in the visual modality (Frégnac, 1996; McLean & Palmer, 1998). Recent evidence shows that *top-*

down processes lead to a *specific* enhanced activation of neurons tuned to conditioned frequencies or an inhibition of neighbouring side-bands (Fritz, Shamma, Elhilali, & Klein, 2003). The experimental modification of sensory maps also accompanies ameliorations in performance as can be seen by discrimination performance in animals (Recanzone, Schreiner, & Merzenich, 1993) or humans (Menning, Imaizumi, Zwitserlood, & Pantev, 2002; Menning, Roberts, & Pantev, 2000). Certainly these observations would remain purely artificial laboratory phenomena, but they are validated by investigation of subjects that are trained to heavily depend on a certain modality such as musicians (Elbert, Candia, Altenmüller, Rau, Sterr, Rockstroh, Pantev, & Taub, 1998; Münte, Altenmüller, & Jäncke, 2002; Pantev, Oostenveld, Engelien, Ross, Roberts, 1998; Schneider, Scherg, Dosch, Specht, Gutschalk, & Rupp, 2002) or due to a certain disability (e.g. Braille-reading in blind subjects; Goldreich & Kanics, 2003; Röder, Teder-Salejarvi, Sterr, Rösler, Hillyard, Neville, 1999; Sterr, Müller, Elbert, Rockstroh, Pantev, & Taub, 1998). As to the purpose of use-induced plasticity, it can be asserted that the processes reflect the attributed behavioural significance of certain stimuli. Conversely it is widely presumed that cortical plasticity *can not* be spurred without behavioural significance of the stimulus (Ahissar, Abeles, Ahissar, Haidarliu, & Vaadia, 1998; Ahissar & Ahissar, 1994; Ahissar, Vaadia, Ahissar, Bergman, Arieli, & Abeles, 1992; Pantev, Weisz, Schulte, & Elbert, 2003; see also section 1.2.4.). The possibility to modify response properties of neurons by behavioural intervention open up new perspectives in the field of neurorehabilitation (Elbert & Heim, 2001; Taub, Uswatte, & Elbert, 2002).

Following a damage to peripheral receptors, receptive fields of neurons deprived of their normal input shift toward undamaged lesion-edge regions (Rauschecker, 1999). This mechanism – observable in all modalities – is very important for the ideas concerning tinnitus in this thesis, thus a description for the auditory modality is given in an separate section (see section 1.2.2.). Pioneering work in this field was accomplished by Merzenich and colleagues (Merzenich, Kaas, Wall, Nelson, Sur, & Felleman, 1983; Merzenich, Nelson, Stryker, Cynader, Schoppmann, & Zook, 1984) after median nerve transaction or amputation of digits in monkeys: deafferented neurons become responsive to stimulation of neighbouring skin areas, that are represented in neighbouring zones on a somatotopic map. That cortical reorganization can occur on a very large scale was demonstrated by Pons and

colleagues (Pons, Garraghty, Ommaya, Kaas, Taub, & Mishkin, 1991). Clinically of interst, somatosensory map reorganization is claimed to underly phantom-limb pain following amputation (Elbert, Flor, Birbaumer, Knecht, Hampson, Larbig, & Taub, 1994; Flor, Elbert, Knecht, Wienbruch, Pantev, Birbaumer, Larbig, & Taub, 1995; Ramachandran, 1993). Some authors propagate sensory phantom phenoma to be a general consequence of map plasticity (Irvine, Rajan, & Brown, 2001; Rauschecker, 1999): **from this perspective phantom limb pain as well as tinnitus may constitute perceptual 'garbage' effects of map reorganization that follows deafferentation**, as they obviously do not serve a specific purpose. The analogy between tinnitus and phantom pain was also the basis for the work on tinnitus by Mühlnickel et al. (1998), which stood at the origin of tinnitus research in Konstanz. We will return to this issue later on (section 1.2.3.).

One process by which the described events have been explained is that a damage to peripheral receptors disturbs an existing balance between excitation and inhibition in the respective deafferented cortical areas: i.e., these areas loose their normally inhibitory influences on thalamocortical or cortico-cortical afferents from neighbouring areas. This leads to an overrepresentation of the perilesional sensory epithelia. Loss of surround inhibition is probably involved in so called 'filling-in' phenomena by which the brain completes missing sensory information as in the case of scotomas or illusions (see e.g. Pessoa & De Weerd, 2003). Analogously tinnitus has been sometimes viewed as an auditory filling-in phenomenon (e.g., Kadner, Viire, Wester, Walsh, Hestenes, Vankov, A., & Pineda, 2002; Norena, Micheyl, & Chery-Croze, 2000; Pantev et al., 2003). This approach to tinnitus will be discussed in an upcoming section. All in all however, impairment of lateral inhibition occurs almost immediately (Pantev, Wollbrink, Roberts, Engelien, & Lütkenhöner, 1999), excluding the possibility for 'real' plastic processes. The current understanding of neuroplasticity spots the elementary mechanisms on a synaptic level, i.e. in a change of communication between neurons particularly on a horizontal level (i.e., cortico-cortical; Buonomano & Merzenich, 1998). Synaptic efficacy between neurons is modulated in a use dependent way following a Hebbian learning rule (Hebb, 1949), which states that connections between neurons that exhibit correlated pre- and postsynaptic activity are strengthened. Experimental data on a cellular level supporting this view stem particularly from studies on long-term potention (LTP; and

also its counterpart long-term depression, LTD) which has been mainly investigated in the hippocampus with a special respect to learning and memory: repetitive activation of an excitatory synapse leads to an increase of synaptic strength (i.e., it becomes potentiated; see e.g. Malenka & Nicoll, 1999). LTP is critically dependent on the activation of N-methyl-D-asparate (NMDA) receptors following depolarization of the neuron, allowing an influx of Ca^{2+}. The potentiating effect presumably stems from increasing the function or number of glutamergic AMPA receptors. Its features of specifity and associativity (Malenka & Nicoll, 1999) make it a candidate mechanism for describing use-induced plasticity. NMDA antagonists that block LTP are also known to prevent map reorganization following deafferentation (reviewed in Buonomano & Merzenich, 1998). Thus injury-induced plasticity may share some of the cellular and molecular mechanisms underlying LTP.

1.2.2. Reorganization of the auditory cortex after peripheral damage

It has been mentioned earlier that insights from injury-induced plasticity research has been pivotal for the development of the research approach presented in this thesis. So what happens in auditory cortical areas after functioning of hair cells in the cochlea is detrimented, e.g. by noise, ototoxic agents or simply by aging (presbyacusis)? And what implications does this have for tinnitus? Clearly, the second question is more difficult to answer and an all-embracing explanation can not be given here due to lack of knowledge and consensus. **Generally most reasearchers assume that the injury has to exceed a certain threshold and that hearing losses have to be steep rather than gradual to induce cortical reorganization**. Out of the two variables however, slope is the more significant variable (Rajan, 1998). Interestingly, **enhancements of frequency-discrimination limens at audiometric edge frequencies seem to be largely restricted to steeply sloping hearing losses** (Thai-Van, Micheyl, Moore, & Collet, 2003; Thai-Van, Micheyl, Norena, & Collet, 2002).

Various studies were able to show that frequency tunings of neurons in the auditory cortex shift towards perilesional edge receptors, leading to an overrepresentation of these receptors and their associated characteristic frequencies (Irvine et al.; Rajan & Irvine, 1998; Syka, 2002). These effects have been observed in several species such as cats (Rajan, Irvine, Wise, & Heil, 1993), mice (Willott, 1996)

and macaque monkeys (Schwaber, Garraghty, & Kaas, 1993). Thus it can reasonably be assumed that similar processes occur in humans too (Dietrich, Nieschalk, Stoll, Rajan, & Pantev, 2001). These studies are especially important, as **they indicate that normal hearing thresholds do not mean normal neuronal processing of such frequencies**. Furthermore acoustic information in the audiometrically normal region appears to be more salient at least in mice as shown by Willott and colleagues (Willott & Carlson, 1995; Willott, Carlson, & Chen, 1994). Tones presented in this frequency region lead to an enhanced prepulse inhibition. It remains an open issue right now, to what extent reorganization is a cause or effect (or both) of an enhanced salience.

Besides of map reorganization, spontaneous activity of neurons in the auditory system is altered too after damage of peripheral receptors. This is viewed by some as neural code of tinnitus. Enhanced spontaneous activity was observed e.g. in subcortical structures such as the inferior colliculus (Salvi, Wang, & Ding, 2000) or the dorsal cochlear nucleus (Brozoski, Bauer, & Caspary, 2002; Kaltenbach & Afman, 2000; Kaltenbach, Zacharek, Zhang, & Frederick, 2004; Rachel, Kaltenbach, & Janisse, 2002). Other authors report enhanced spontaneous activity in deafferented regions of the primary auditory cortex following treatments (e.g., noise, salicylate, quinine) that presumably induced tinnitus in animals (Eggermont & Komiya, 2000; Komiya & Eggermont, 2000; Norena & Eggermont, 2003). Greater spontaneous firing rates in animal tinnitus subjects have also been observed in the secondary auditory cortex (Eggermont & Kenmochi, 1998). Next to an increased firing rate Norena and Eggermont (2003) also found greater correlated activity of such neurons (see also Ochi & Eggermont, 1997).

Enhanced spontaneous activity and map reorganization after deafferentation might share similar mechanisms. Considering use-induced plasticity, Hebbian learning is regarded as a fundamental principle for cortical reorganziation. For injury-induced plasticity Buonomano and Merzenich (1998) proposed a competetive mechanism (postsynaptic normalization) according to which the total synaptic input to a cell remains constant. That means reduction of input from one synapse will cause enhanced input from other synapses. An activation of sensory intact receptors is also suggested as a driving force for map reorganization (Weinberger, 1998), especially when there is an increase in the behavioural significance of such input. Another aspect was discussed above, that neurons that lack peripheral input exhibit a decreased

property to inhibit excitatory influences from neighbouring neurons (Irvine et al., 2001). Both mechanism may play a role in tinnitus on different temporal scales: following a hearing impairment the almost instantaneous loss of surround inhibition could be responsible for the rapid onset of tinnitus sensation frequently reported by patients. Decrease of inhibition is e.g. induced during temporary threshold shifts following noise (Calford, Rajan, & Irvine, 1993; Chermak & Dengerink, 1987). For longer lasting reorganization and thus for tinnitus to become chronic, permanent changes to synaptic connections in affected regions of the auditory cortex seem to be necessary. Towards the end of this thesis a mechanism will be proposed that could contribute to the manifestion of cortical reorganization following damage to auditory receptors (map reorganization as well as spontaneous activity) by integrating knowledge from psychoacoustics with principles from use-induced plasticity.

1.2.3. Reorganization of the auditory cortex and its potential relation to tinnitus

If map reorganization and altered spontaneous activity take place within the same deafferented regions, what can be regarded as more relevant in the case of tinnitus? The answers given by scientists are not uniform and the alternatives are usually not seen as mutually exclusive. However in most cases a preference concerning an explanation can be identified.

Map reorganization

Oversimplified, the faction emphasizing map reorganization draw an analogy between tinnitus and neuroplastic processes associated with phantom pain (Rauschecker, 1999). In the latter patient group representational areas of body parts next to the deafferented region on the somatotopic map expand (Elbert et al., 1994; Flor, 2002; Flor et al., 1995; Ramachandran, 1993; Rauschecker, 1999). Moreover the amount of phantom pain is positively correlated with the extent to which the deafferented region is 'invaded' by neighbouring regions (Flor et al., 1995). Applied to tinnitus, a cochlear damage would resemble an auditory amputation. **This approach would state that an overproportionally large neuronal patch on the tonotopic map is tuned to a lesion-edge frequency region which is seen as a kind of 'tinnitus frequency' area** (Rauschecker, 1999). So far the only study claiming to have experimentally proven the phantom limb analogy for tinnitus was conducted by

Mühlnickel et al. (1998). The authors reported a significant deviation of sources for the 'tinnitus frequency' from the expected location on the tonotopic map. Similar to the result in phantom pain sufferers, the strength of the tinnitus sensation was strongly correlated with cortical reorganization (r = .82; see Figure 1-2). Irvine et al. (2001) have however rightly criticized that this correlation is completely driven by four subjects with extreme reorganization. The neurophysiological data of the remaining six is well in the normal range. Also to eliminate feared confounding influences of hearing loss these scientists excluded participants with a hearing loss above 25 dB HL. Following the analogy to phantom limb pain however it would have been logical to investigate subjects with at least moderate hearing loss (> 25 dB HL), besides questions concerning the representativity of such a sample.

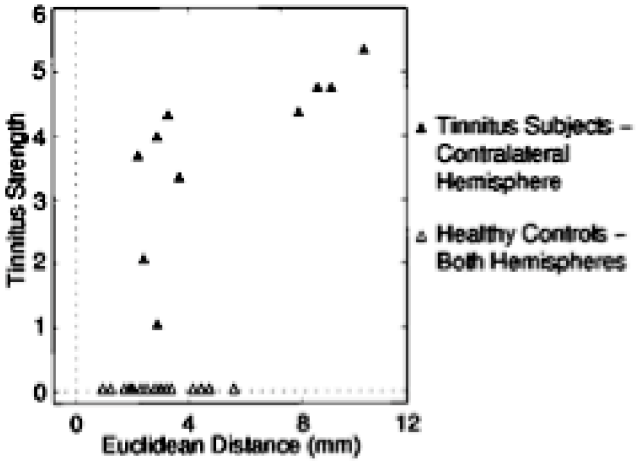

Figure 1-2: Display of the main result of Mühlnickel et al. (1998), i.e. the positive association between rated tinnitus strength and deviation from expected location on the tonotopic map. However, the fit is strongly determinated by four tinnitus sufferers with extreme values.

In a recent study published by Diesch, Struve, Rupp, Ritter, Hülse and Flor (2004), the representativity problem was overcome by including tinnitus sufferers with hearing loss (six of ten subjects with HL > 25 dB HL). Several test frequencies were determined based on the audiometric edge frequency and the 'tinnitus frequency' which consistently lay inside of the hearing loss. Of the several components the authors attempted to investigate within one experiment (N1m, Pbm, SSF, MMF), they were able to show a decrease of amplitude of the steady state field (SSF) with increasing test frequency until the audiometric edge and an increase again towards the 'tinnitus frequency'. The authors interpret this as evidence for an increased SSF in

tinnitus due to a hyperexcitability of neurons for this frequency. Although other studies could show a systematic decrease of amplitude of the SSF with increasing frequency (Ross, Borgmann, Draganova, Roberts, & Pantev, 2000; Weisz, Keil, Wienbruch, Hoffmeister, & Elbert, in press) for normal hearing subjects, it is odd how Diesch et al. could come to such a conclusion without reporting results from a control group. Furthermore, as the 'tinnitus frequency' was located in the hearing loss region, an appropriate control should have been taken regarding recruitment, which could also explain the strong correlations between SSF amplitude and tinnitus intrusiveness (Goebel & Hiller, 1998). It remains highly doubtful whether such complex nonlinear phenomena can be adequately dealt with post-hoc partial correlations. The notion of a hyperexcitible neuronal 'tinnitus frequency' patch was also the basis for the EEG work of Kadner et al. (2002) who demonstrated a stronger (not significant) intensity dependence of the neuronal response (N1) for the 'tinnitus frequency' as compared to controls (tested at 4 kHz). At the same time the intensity function for a 2 kHz probe tone was significantly reduced, while no difference was found for 1 kHz. The authors regard this as support for an enhanced activity in the 'tinnitus frequency' region which causes an inhibition of neighbouring regions (here 2 kHz). A further study belonging to this research approach was conducted by Dietrich et al. (2001), who reported significantly enhanced neuronal response amplitudes for the audiometric edge frequency in subjects with high-frequency hearing loss as compared to two frequencies well in the normal hearing range. This corresponds nicely to predictions made on the grounds of animal studies (Irvine et al., 2001; Rauschecker, 1999), however the authors were unable to find an association with subjective variables of tinnitus. A popular argument against the idea that tinnitus is causally related to map reorganization is that tinnitus sufferers almost never match the 'tinnitus frequency' to the audiometric edge but somewhere inside the hearing loss region (Norena et al., 2002). If the phantom limb analogy is valid however, then this should be expected, as the sensation corresponds to the central representation of the *missing* sensory information. **Overall however, it has to be said that although the seemingly intuitive and plausible analogy with phantom limb pain is appealing, empirical support for the notion is rather scarce and not very consistent**.

Spontaneous activity

Research about the role of altered spontaneous activity has been largely confined to work on animals. The advantage about animal studies is – as usual – the high degree of experimental control and more detailed knowledge about what contributes to the signal measured invasively (a disadvantage especially of EEG and MEG). As tinnitus is a *subjective* phenomenon, the *fundamental* disadvantage of animal studies is the uncertainty whether the animal really suffers under this condition: It will continue to stay an unproven assumption, although some efforts are undertaken to objectify tinnitus by behavioural reactions (Heffner & Harrington, 2002; Jastreboff, Brennan, Coleman, & Sasaki, 1988; Kaltenbach et al., 2004). A good example that work on animals is very useful for generating *ideas* what neuronal processes might be involved in tinnitus is shown by a recent study done by Norena and Eggermont (2003). These authors systematically investigated how different aspects of spontaneous activity (overall firing rate, burst firing pattern, synchrony) and also map reorganization change after loud pure tone trauma with an emphasis on temporal properties. As tinnitus evolves almost immediately due to noise trauma, the assumption is that neurophysiological indicators that change rapidly and remain stable must constitute the neural code of tinnitus. The main result of this study lies in a rapid (~ 1 min post noise exposure) increase in correlated neuronal activity (measured as peak cross-correlation) for above trauma-frequency neurons (especially > 1 octave), which also represent those whose characteristic frequency shifts towards below trauma frequency. Moreover this immediate reorganization was uncorrelated with overall increase in firing rate. **The authors see this as support for the idea of synchronous neuronal activity being the underlying neuronal correlate of tinnitus and that map reorganization might be a mere epiphenomenon of the same underlying process: unmasking of latent inputs**. From this perspective long-term plastic processes only play a subordinate role. It is strange that spontaneous activity so far has not been a real focus of interest in tinnitus research on humans. One exception however lies in studies, attempting to validate low threshold calcium spike bursts as a common neurophysiological correlate for positive symptoms in general. That means that studies (Jeanmonod, Magnin, & Morel, 1996; Llinas, Ribary, Jeanmonod, Kronberg, & Mitra, 1999) were not exclusively dedicated to tinnitus and that the number of tinnitus subjects are rather small. According to this approach

positive symptoms are related to low-threshold calcium spike (LTS) bursts arising in the thalamus as a consequence to cell membrane hyperpolarization (similar to that seen during slow wave sleep; Steriade, 1993). These bursts have a characteristic temporal pattern in the delta to theta range (~2-6 Hz), and this activity is projected via thalamocortical connections to the auditory cortex in the case of tinnitus. A summary of the main concepts is displayed in Figure 1-3.

Therapeutical implications

Currently, various attempts are being made to therapeutically implement notions gained from neuroscientific research. One of these new approaches applies repetitive transcranial magnetic stimulation (TMS) to the primary (Eichhammer, Langguth, Marienhagen, Kleinjung, & Hajak, 2003; Langguth et al., 2003; Plewnia, Bartels, & Gerloff, 2003) or secondary auditory cortex (Plewnia et al., 2003). The basic idea is that tinnitus is a consequence of a hyperexcitability of neurons and that application of TMS leads to a reduction of tinnitus perception due to an inhibition of neuronal activity in these areas. Reports of significant results have to be interpreted with caution, as the samples (Eichhammer et al., 2003; Langguth et al., 2003) or effects are rather small (8 from 14 - 57% - reporting a reduction of tinnitus perception in Plewnia et al. (2003)). Another technique currently tested by some researchers are various forms of frequency discrimination trainings, which attempts to reverse maladaptive injury-related by use-induced plasticity (Elbert & Heim, 2001). Two general approaches can be differentiated. The notion underlying the first one is that due to missing inhibitory mechanisms a 'tinnitus frequency' area is overrepresented in the auditory cortex. The work by Mühlnickel et al. (1998) has been very influential for this approach. By training the subjects in frequency regions neighbouring the 'tinnitus frequency' it is thought that its representation can be downsized and the tinnitus severity diminished. Recently Flor, Hoffman, Struve and Diesch (in press) published their first results on this type of training for 12 participants who trained for four weeks. Half of the sample practiced in a region either close or remote to the 'tinnitus frequency'. Contrary to expectation, the frequency region played no role. Unfortunately, this stands in contrast to the rather euphoric initial announcements that were spread by the media that tinnitus severity can be reduced by 35% with such a training (see e.g. NZZ from 8.12.02; New Scientist, 21.3.02).

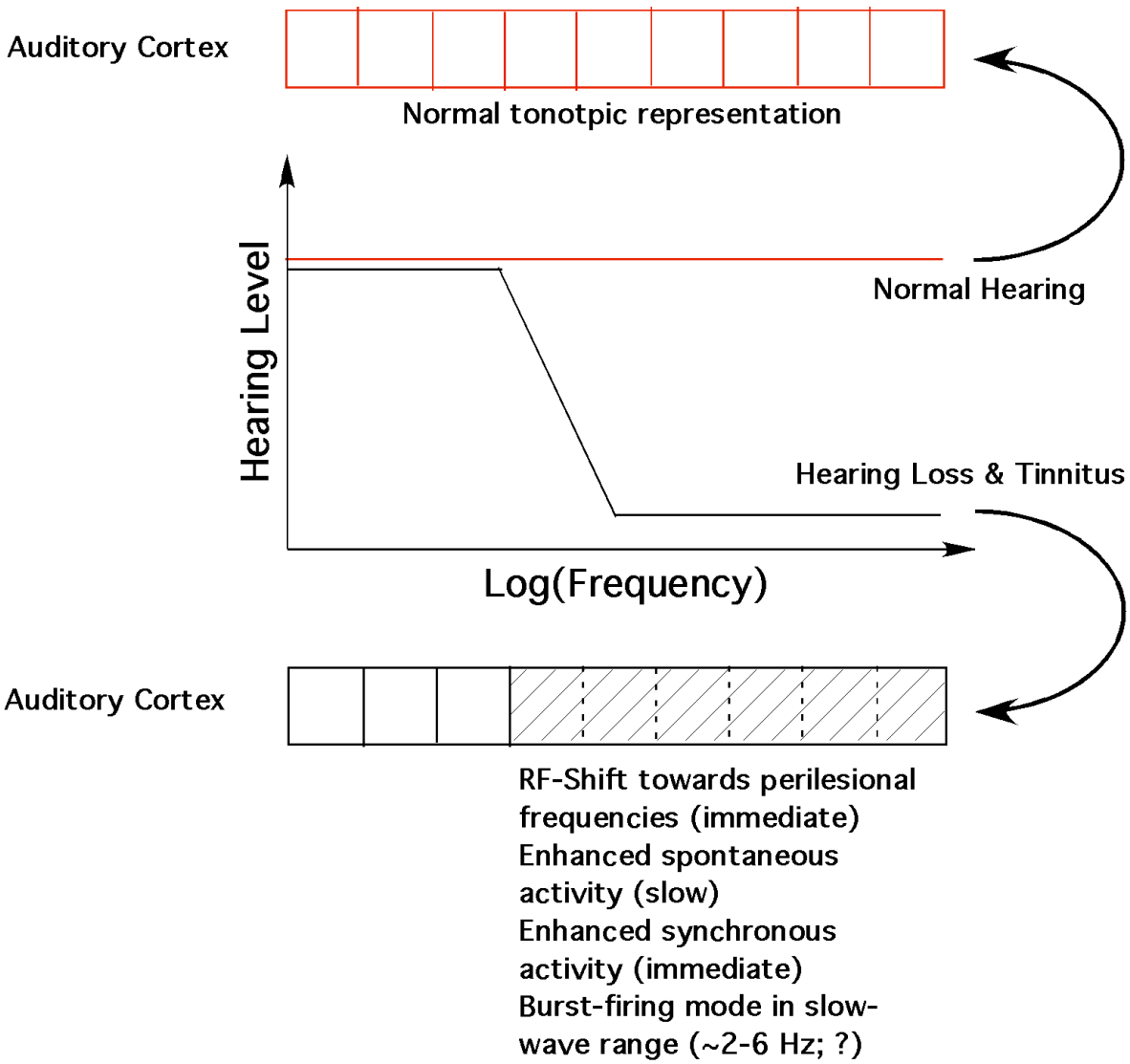

Figure 1-3: A very oversimplified portrayal of the main concepts on auditory cortical reorganization that have been discussed as neurophysiological correlates of tinnitus. The majority of researchers agrees that a hearing impairment is a requirement for the development of tinnitus. This is indicated here as high-frequency hearing loss (black line; red line normal hearing level), which is simply exemplary, i.e. the audiograms vary considerably between subjects (and sometimes there is no audiometric hearing loss). Map reorganization, synchronous firing and enhanced spontaneous activity in deafferented regions (shaded area) have been related especially to unmasking of latent inputs (see e.g., Norena & Eggermont, 2003), while burst firing has been connected to thalamic low-threshold spike bursts projecting to the auditory cortex (Jeanmonod et al., 1996).

The only effect reported was a 'dose-effect': subjectively reported tinnitus severity got worse for participants training irregularly (tinnitus severity scale 0-9; pre: 6, post: 7.5) while it got better for the group training regularly (pre: 4.5, post: 4). The increase in the irregular training group amounts to 25% while the corresponding decrease in the regular training group is 11%. Taken together tinnitus severity increased from approximately 5.1 to 5.5. Based on these results it seems obscure how the authors can conclude that their treatment "has a positive effect on tinnitus severity and related variables". A related approach focuses on the hearing loss itself rather than the 'tinnitus frequency' which constitute areas with loss of surround inhibition leading to map reorganization and altered spontaneous activity. This approach is tested by some researchers (Norena et al., 2002) and is also favoured by the group in Konstanz. So far there is no evidence that a frequency discrimination training inside of the hearing loss region leads to any substantial reduction of tinnitus perception. In the context of a working model how peripheral input might influence cortical reorganization (see Discussion) I will discuss a possible explanation why the results of frequency discrimination trainings are unsuccessful so far. Overall, although many valuable insights have been gained from basic research on neuroplasticity and tinnitus the impact on therapeutic strategies has been rather weak.

1.2.4. Behavioural relevance

One point neglected up to now is that cortical reorganization is most effectively triggered, when the overall context is important for behaviour and experience (in the following only refered to as *behavioural relevance*). Stimulation embedded in a discrimination task (Recanzone, Merzenich, Jenkins, Grajski, & Dinse, 1992; Recanzone et al., 1993) or conditioning paradigm (Edeline et al., 1993; Edeline & Weinberger, 1993; Weinberger, 1998) leads to an enlarged representation of the relevant stimuli. Important however is that passive stimulation does not lead to a similar reorganization (Ahissar et al., 1998; Recanzone et al., 1992; Recanzone et al., 1993). Yet, recently Pleger, Dinse, Ragert, Schwenkreis, Malin and Tegenthoff (2001) reported ameliorated discrimination performance and altered dipole locations of the somatosensory evoked potential (N20) following extensive correlated passive tactile stimulation. Furthermore, the amount of performance gain could be predicted from the neurophysiological data. So it is still debated whether behavioural relevance is a

mandatory condition to induce plastic changes, but its *modulatory* influences are undisputed. For the auditory system, top-down influences are implicated by an extensive corticofugal system, i.e. cortical reorganization leads via efferent fibers to a reorganization of subcortical areas which in turn boosts cortical plasticity (termed '*egocentric selection*'; Gao & Suga, 1998; Gao & Suga, 2000). To what extent reorganization occurs is heavily dependent on activity of the basal forebrain cholinergic system, which is known to be involved in attention and learning. Kilgard and Merzenich (1998) for example were able to show extensive plastic changes of the primary auditory cortex by pairing an acoustic stimulus with stimulation of the nucleus basalis. In another work stimulation of dopaminergic neurons in the ventral tegmental area of rats together with an auditory stimulus lead to an enlarged representation of this stimulus in the primary auditory cortex (Bao, Chan, & Merzenich, 2001). Additionally, the authors were able to observe the emergence of a responsive field outside of the primary auditory cortex. **From the animal works it can thus be expected that *attentional and emotional factors should influence neuroplastic processes* following hearing damage and *might alter the salience of acoustic stimulation in general*** (Willott, 1996; Willott et al., 1994). Indeed, induction of a hearing damage presumably leading to tinnitus in gerbils triggers wide-spread reorganization also in non-auditory limbic system areas – among others frontal cortex, amygdala and hypothalamus – as measured via c-fos expression and 2-deoxyglucose uptake (Wallhäusser-Franke, Braun, & Langner, 1996; Wallhäusser-Franke, Mahlke, Oliva, Braun, Wenz and Langner, 2003).

An indicator that for the distress associated with tinnitus, other aspects have to be involved as well apart from auditory cortical activity seems obvious when considering that there is apparently no consistent association between psychoacoustic features of tinnitus and the level of felt distress (Henry & Meikle, 2000). Most studies concerning possible top-down influences were conducted using neuroimaging methods (fMRI, PET). But there also have been a few EEG studies that implicated e.g. enhanced early selective auditory attention in tinnitus sufferers measured by the early negative difference wave (Nd; Jacobson, Calder, Newman, Peterson, Wharton, Ahmad, 1996) or a habituation deficit in tinnitus 'complainers' (compared to 'noncomplainers' and controls; Walpurger, Hebing-Lennartz, Denecke, & Pietrowsky, 2003). The latter interpretation was derived from a reduced amplitude decrease of the

N1-P2 complex across trials. Neuroimaging studies, in which tinnitus sensation was somehow modulated (e.g., via oral-facial movements), suggest the involvement of non-auditory brain regions in a tinnitus related neural network, e.g. hippocampus (Lockwood et al., 1998) or middle and dorsolateral prefrontal cortex (Mirz, Gjedde, Sodkilde-Jrgensen, & Pedersen, 2000; Mirz, Pederson, Ishizu, Johannsen, Ovesen, Stodkilde-Jorgensen, & Gjedde, 1999). Frequently there is a right sided dominance of effects (Mirz et al., 1999; Reyes et al., 2002), thus an interesting aspect would be to find out more about the role of possible influences of hemispheric asymmetry. This has not been a matter of systematic investigation so far.

A increasingly popular therapeutic approach explicitly proposing a vicious circle of negative emotional states and the inability to habituate to the tinnitus sound is the so-called Tinnitus Retraining Therapy (TRT; Jastreboff & Jastreboff, 2000). This approach is a mixture of methods, with its core elements being an extensive counseling and a low-level broad band noise generator, with the goal of a habituation to tinnitus (i.e., treatment of distress caused by tinnitus rather than the cause of tinnitus). The whole procedure takes approximately 1.5-2 years. Claims of improvements in 75% of cases is certainly exaggerated due to the several methodological deficits of the studies (Kroener-Herwig, Biesinger, Gerhards, Goebel, Greinel, & Hiller, 2000). However it seems clear from the studies cited above that if tinnitus has anything to do with auditory cortical reorganization then any successful therapeutic approach will somehow have to address the handling of adverse top-down influences.

1.3. Temporary summary

In this first part of my thesis the necessary background has been given in order to understand the approach that I have taken. The major aspects can be summarized in the following points:

- The tinnitus generating signal is rarely generated in the periphery but is a consequence of a reorganization of the central auditory system.
- The neuroplastic processes are probably triggered by a hearing impairment, e.g. a damage to receptors of the inner ear. This leads on a short time-scale to a discontinuation of lateral inhibition in the deafferented regions. In the long

run LTP-like alterations of synaptic strengths or formation of new connections between neurons could be involved.

- Candidates for neural correlates of tinnitus are especially map reorganization (especially inspired by works on phantom-limb pain) and changes in spontaneous activity (e.g., overall, synchronous or bursting activity).
- An alteration in salience of acoustic information and the behavioural relevance of the tinnitus sound itself could enforce maladaptive neuroplastic processes.

2. General Methods

This part is intended to give the reader some details about the general methods of the studies presented in the subsequent chapter. Methods *specific* to each study (e.g. design, stimuli etc.) will be described later together with these studies.

2.1. MEG / EEG recording apparatus

MEG data were recorded (sampling rate: 678.17 Hz; 0.1 – 200 Hz analogue filter) using a 148 channel whole-head magnetometer (MAGNES 2500 WH, BTi, San Diego, CA). Before each actual experiment the head shape of each participant was digitized. Additionally, five index points (left and right preauricular points and three locations on the forehead) were determined, to calculate the relative head position within the MEG helmet.

Neuroelectric signals were recorded (A/D rate: 500 Hz; filter DC-100 Hz; SynAmps amplifier, Neuroscan) from 65 locations on the scalp with Ag/AgCl electrodes mounted on an electrode cap (electrode impedances < 5 kΩ). At the end of the EEG-measurement electrode positions, relative to anatomical landmarks of the head, were digitized with a position indicator system (ISOTRAK II, Polhemus).

In both cases vertical and horizontal eye-movements (EOG) were measured from above and below the eye and from the outer canthi for the purpose of offline eye artefact rejection and correction.

2.2. Artefact correction

In all studies artefact correction was performed offline using BESA2000 (version 4.2.26 MEGIS). For this purpose, in a first step the raw data was corrected from blinks using the algorithm (Multiple source eye correction) proposed by Berg and Scherg (1994). This method estimates and corrects for eye-activity in EEG and ERP data in the presence of overlapping brain activity. Eye movement data was selected directly from the continuous data stream of the experimental runs in case of MEG. In the EEG study a separate calibration trial, in which the participant was requested to do eye-movents in specified directions, preceded the actual experiment. In a second step, left over epochs contaminated by artefacts were either rejected per

visual inspection (using BESAs artefact scanning tool; study 2 & 3) or using a threshold criterium (> 2 pT; study 1).

2.3. Source analysis

Data obtained by MEG and EEG does not offer direct information about the location of the neuronal generator(s) that lead to the measured signal changes. Yet several attempts have been made to deal with this inverse problem and by this means to make inferences about the underlying source space activity. Independent of the approach, source analysis always requires a sufficient spatial sampling, i.e. a large amount of electrodes / sensors. In the upcoming two sections a description of the two approaches will be given, that were commonly used in the studies presented here. An important concept for both methods is that of a *dipole*. This reflects the fact that neighbouring neurons (e.g., in a field of the auditory cortex) with a similar orientation exhibit a similar direction of the flow of intracellular currents (Elbert, 1998). When a large amount of neurons is activated in a synchronous manner in a certain region (e.g. in the auditory cortex after an acoustic stimulus), the total activity can be modeled by a so called *equivalent current dipole*. That means that no matter which method (inverse solution) is applied, it should be kept in mind that source modeling in the context of MEG / EEG refers to spatial and temporal properties of massed activity.

2.3.1. Multiple equivalent current dipole fitting approach

The first approach, as implemented e.g. in BESA, assumes that the EEG / MEG signal measured at an electrode / sensor is the weighted sum of activities at several equivalent dipoles at different locations (Scherg, 1991). The influence on the neuroelectric signal outside of the head is not only dependent on the proximity of electrode / sensor to the source but also on the temporal activation pattern and orientation of the dipole. If neuronal activity is expected in different brain regions (each with a similar orientation), then this activity can be modeled by assuming multiple dipoles, the number corresponding to the amount of active brain regions. The amount of active source that can be assumed however is *maximally limited to the number of electrodes / sensors* (i.e., overdetermined equation system). Other than with a *moving dipole*, in which location and orientation of the dipole varies with time making the interpretation of the dynamics of source activity difficult, fixed source

locations and orientations were employed here (*spatiotemporal model*; Scherg & Von Cramon, 1986). The only parameter allowed to change as a function of time is the strength of the dipole. Mathematically the source problem can be formulated as finding a solution for:

$$(1)\ U = C * S,$$

where U is a matrix with the measured electric potential / magnetic field for each electrode / sensor (k) as a function of time (i.e., $u_k(t)$). C is a matrix with constant (i.e., time-invariant) weighting coefficients, which are a function of the location and orientation of a source j and the position of k (i.e., c_{jk}). S represents the strength of j as a function time (i.e., $s_j(t)$). The optimal solution (in a least-square sense) to this problem is then determined by the pseudoinverse of C (C^p) which can be applied to (1), to yield predicted (model) potentials / magnetic fields U' (forward solution). The deviance from the observed data can then be either expressed as norm of the difference matrix |U' – U| or as residual variance / goodness of fit. If location and orientation of the dipole(s) are unknown then they can be fitted by iterative procedures which successively alter the parameters of the model until the residual variance reaches a minimum. For physiological and mathematical details concerning this approach, the reader is refered to Scherg and von Cramon (1986) or Hämäläinen, Hari, Ilmoniemi, Knuutila and Lounasmaa (1993).

As cortical areas can be significantly convoluted within small spatial extents, so-called *regional sources* (Scherg & von Cramon, 1986) were employed in two studies presented here. Basically, these sources correspond to two (in the case of MEG) or three (in the case of EEG) spatially identical dipoles with orthogonal orientations. In both studies (2 & 3) using this approach a time window of 30 ms around the peak of the N1m was used to fit the location and orientation of two dipoles under the constraint that the location should be symmetric. In study 3 a fixed source model was employed that acted stronger as a kind of spatial filter for auditory cortical activity. To conclude: the approach described here seems especially appropriate when the focus of neuronal activity is known. In the context of the work presented in this thesis, this was specifically the case for studies 2 and 3, where participants passively listened to acoustic stimuli while being engaged in reading a book of their choice (study 2) or watching a movie without sound (study 3). Under these circumstances it is plausible to expect dominant auditory cortical activity.

2.3.2. Minimum Norm Estimate

A further linear estimation technique used especially in study 1 is the so-called minimum norm estimate (MNE; Hämäläinen & Ilmoniemi, 1994), for which in-house software exists programmed by Hauk (Hauk, Keil, Elbert, & Müller, 2002; for explorative purposes however, study 2 employed BESA). Differing from the approach described above, where the number of sources has to be fitted (location, orientation, strenght) under the constraint that they do not exceed the number of electrodes / sensors, the prerequisite for the MNE is that the amount of sources lies above that of the electrodes / sensors (underdetermined equation system). The sources (1384) are fixed in location (with 2 orthogonal, tangential orientations in case of MEG, corresponding tp 2768 dipole components; an additional third radial orientation in the case of EEG) and distributed on three concentric shells that is intended to account for varying depths on neuronal activity (80%, 60% and 40% of head radius; in order to average MNE solutions over participants head radius was set to a constant value of 10 cm, see also Moratti, Keil, & Stolarova, 2004). Similar to the approach above MNE assumes that the observed data U at a specific sensor is determined by the current density of the sources S, the sensor's sensitivity to the various sources (the so-called lead-field matrix, L) and a noise component ε:

$$(2)\ U = LS + \varepsilon$$

For the estimation of S the L2-norm was used which minimizes the squared current density, which is obtained by multiplying the pseudoinverse of L with the observed data. In order to obtain stable solutions, spatial regularization needs to be performed with a regularization parameter λ. The method used in the in-house software is the Tikhonov-Philips regularization, which is applied during pseudoinversion of the leadfield matrix. Data reported in study 1 refer to the solution for shell 2 (6 cm radius), for which Hauk et al. (2002) were able to show an optimal tradeoff between blurring (i.e., poor spatial resolution) and depth sensitivity. The big advantage about MNE is that mathematically unique solutions can be obtained without the need to specifiy a priori the number and location of sources. Thus this distributed source estimation method seems appropriate in cases when exact information about possible source configurations are lacking, which was specifically the case for study 1.

2.4. Hearing thresholds and test determination of frequencies

Hearing thresholds in dB HL were assessed with a clinical audiometer AC40 (InterAcoustics) and TDH 39p headphones (Telephonics). Standard test frequencies were 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 16 kHz. For all tinnitus participants – everyone exhibiting at least a mild high-frequency hearing loss (i.e., > 25 dB HL), an effort was made to sample more frequencies in the region where normal hearing thresholds progressed into hearing loss thresholds, i.e. to measure out the hearing loss slope (see Figure 2-1). The intention of this procedure was to determine a lesion-edge frequency for each tinnitus sufferer. This value was defined by visual inspection as the audiometrically normal start frequency of the hearing loss slope. The lesion-edge frequency and a control frequency (set at an octave below the lesion-edge) play particularly a role in studies 2 and 3.

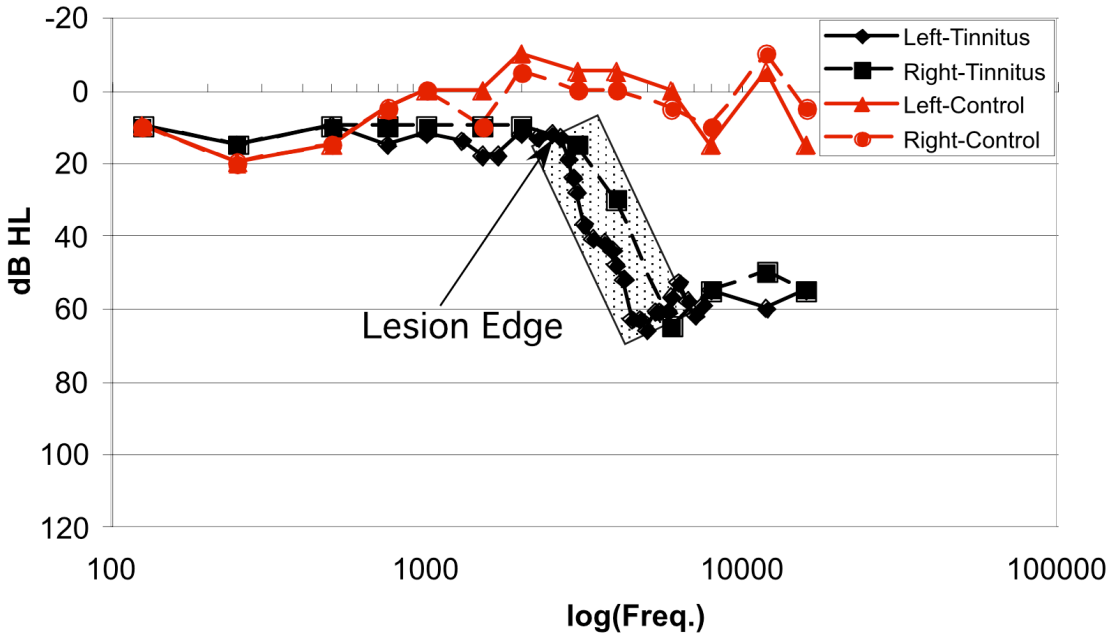

Figure 2-1: Example audiograms (range 0.125-16 kHz) of one representative tinnitus (black lines and symbols) and normal hearing control subject (red lines and symbols). The latter person exhibits thresholds close to the 0 dB HL line. For the tinnitus subject thresholds are normal up to approximately 2 kHz and then commence to decrease in a relatively linear manner, reaching a plateau at about 4 kHz.

Depth of hearing loss was defined as the absolute difference between the threshold (in dB HL) at LE to the steepest point of the hearing loss slope. Steepness of the hearing loss slope was defined as the regression-coefficient of following formula:

$$h \sim \boldsymbol{a}^{*}(\log_{10}(f)) + b,$$

where *h* is the hearing threshold, *a* is the slope (corresponding to steepness of hearing loss), *f* is the relevant frequency in the audiogram and *b* the intercept.

2.5. Assessment of tinnitus related distress

In order to quantify tinnitus related distress, we employed the Tinnitus Fragebogen (TF, Goebel & Hiller, 1998; adaption of the Tinnitus Questionnaire by Hallam, Jakes, & Hinchcliffe (1988)) which represents a standard questionnaire for this purpose in Germany. It assesses distress on multiple scales:

1. emotional distress (maximum score: 24)
2. cognitive distress (16)
3. emotional and cognitive distress (40): obtained by adding scores on 1 and 2
4. intrusiveness (16)
5. auditory perceptual disturbances (14)
6. sleep disturbances (8)
7. somatic complaints (6)

The manual offers rules of thumbs to classify patients according to their score on the questionnaire (maximally 84 points):

- minor distress: 0 – 30
- moderate distress: 31 – 46
- severe distress: 47 – 59
- very severe distress: 60 – 84

3. Experiments

In total three studies with their aims, main results and discussion will be presented. In chapter 4 I will attempt to discuss all three studies in a more general and unified manner. This part corresponds strongly to manuscripts that have been published (study 2, Weisz, Voss, Berg, & Elbert, 2004a), submitted (study 1) or are in preparation for submission (study 3).

One study (study 1) intended to explore potentially abnormal spontaneous activity patterns especially in the low-frequency range (< 4 Hz). Two studies follow the notion of seeing tinnitus as auditory phantom pain analogy and expecting specific effects for lesion-edge frequencies (study 2 and 3) due to map reorganization, a notion that stands at the origin of the tinnitus project in Konstanz. An unexpected negative correlation between hearing loss slope and tinnitus distress was also observed and is presented as a side effect in the last section of this chapter.

In the course of this dissertation, two basic studies were concerned with the neuromagnetic assessment of tonotopic representation in humans. The main message of these studies was that even though tonotopy is likely to be measurable on a group level using MEG, the high interindividual variability make these methods inappropriate for the present purpose: i.e., to determine small deviations (few mm) from ordinary tonotopic representation. For the reader interested in details of the studies two manuscripts are added in the appendix (A and B). These manuscripts were recently accepted for publication (Weisz et al., 2004b; Weisz, Wienbruch, Hoffmeister, & Elbert, 2004c).

3.1. Study 1: Spontaneous Activity

3.1.1. Aim

Most animal studies on tinnitus concentrated on aspects of spontaneous activity. In humans this has been largely neglected so far: here, neuronal responses following acoustical stimulation or modulations of tinnitus loudness usually formed the focus of interest. It has to be emphasized that talking about spontaneous activity in animal and human studies does not correspond to exactly the same issue. In animal studies an invasive investigation offers the possibility to measure the firing pattern (action potentials) of neurons (single or multiple unit), which is not possible with

MEG and EEG. However a synchronous activation of a large number neurons will also generate a measurable signal at the surface of the head, provided that the activation is strong enough and not too distant from the scalp (i.e., both methods are especially sensitive to cortical sources). This synchronous activity is usually characterized by various temporal patterns, that often correspond to different cognitive, wakeful or clinical states. This study attempted to investigate whether abnormal neuromagnetic spontaneous activity patterns can be observed in a resting condition. A focus was especially put on slow-wave activity in the delta (< 4 Hz) and theta (~4-8 Hz) range. One reason for this was that tinnitus as a 'positive symptom' has been specifically related to thalamic low-threshold calcium spike bursts (see section 1.2.3.; Jeanmonod et al., 1996; Llinas et al., 1999) that should lead to a synchronous slow wave activity in auditory cortical areas due to thalamocortical projections. It seems as deafferentation in general is associated with an enhanced slow wave activity (specifically delta), as can be observed in the presence of neurological damage (de Jongh, de Munck, Baayen, Jonkman, Heethaar, & van Dijk et al., 2001; Kamada, Moller, Saguer, Ganslandt, Kaltenhauser, Kober, & Vieth, 2001; Vieth, Kober, & Grummich, 1996) or slow wave sleep (Benoit, Daurat, & Prado, 2000; Gath & Bar-On, 1983). Furthermore enhanced activity in the delta frequency range is known to be frequently accompanied by a reduction of alpha power (~8-12 Hz; Benoit, Daurat, & Prado, 2000; Canive, Lewine, Edgar, Davis, Miller, Torres, & Tuason, 1998; Canive, Lewine, Edgar, Davis, Torres, Roberts, Graeber, Orrison, & Tuason, 1996; Gath & Bar-On, 1983; Uchida, Maloney, March, Azari, & Feinberg, 1991).

3.1.2. Methods

Participants: Seventeen chronic tinnitus sufferers (one woman; age: 52.41 ± 2.70) with hearing loss (16 cases high frequency hearing loss; 1 case deaf on left ear; 3 cases normal hearing on right ear) and 16 normal hearing control subjects (one woman; age: 45.88 ± 3.84) participated in the study. Tinnitus was reported to be bilaterally equal in 4 cases, bilateral but left dominant in one case and right dominant in 2 cases. Ten cases stated to have unilateral tinnitus from which 8 cases indicated that they hear their tinnitus on the left side. Prior to the experiment participants gave written informed consent. Tinnitus related distress was assessed with a standard

German questionnaire (Tinnitus Fragebogen, TF; Goebel & Hiller, 1998). Next to the total score (sum of scores on each subscale), following subscales were analyzed separately: emotional distress, cognitive distress, intrusiveness and sleeping problems. The latter subscale, together with the regional slow wave distribution should help to rule out that changes in frequency bands could be a trivial result of sleep disturbances.

Data acquisition: Five minutes of MEG under a resting condition were recorded. The participant was requested to keep eyes open and to maintain gaze on a fixation mark at the ceiling of the recording chamber.

Data reduction and analysis: In a *first step* of data analysis, sampling points were reduced by a factor ten. As an explorative step, spectral power was calculated for each sensor via mean FFTs (window length: 7.55 sec; 50% overlap between cosine squared windows). This was done to focus on specific frequency-bands of interest. As the emphasis of the study was on alterations of spontaneous activity patterns within the tinnitus group, data was scaled by dividing each value by the overall mean power (gained by averaging over all sensors). As depicted in Figure 3-1, tinnitus sufferers show a markedly reduced alpha peak accompanied by an enhancement in the slow frequency (delta) range. The *second step* consisted in the investigation of the underlying source activity via application of the Minimum Norm Estimate (MNE, see section 2.4.3.; Hämäläinen et al., 1993; Hauk et al., 2002) to the eye-movement corrected continuous data. Each of the 197 dipole locations consisted of two perpendicular dipoles oriented tangentially to the shell surface. The source-space transformed continuous data was then entered to the same mean FFT algorithm as described above. For both orientations of each dipole, power was calculated in the following frequency bands: delta (1.5-4 Hz), theta (4-8 Hz) and alpha (8-12 Hz). In order to obtain a single value for each dipole the square root of the sum of squares of the power for the two orientations was calculated and scaled in the same manner as described above (using mean power of each dipole instead of each sensor). Oscillatory activity was then analyzed a) averaged over all 197 dipoles and b) in specific regions by grouping clusters of dipoles on the sphere and averaging their values. These regions (always bilateral) were: prefrontal (RPF, LPF), frontal (RF, LF), temporal (RT, LT), parietal (anterior: RPA, LPA; posterior: RPP, LPP), and occipital cortex (RO, LO).

Statistical analysis: We employed repeated-measures ANOVA to test for the presence of main and interaction effects to the overall average for each frequency. Since the result of this analysis was significant we executed this analysis for specified regions separately. Associations with tinnitus-related distress were tested using product-moment correlations. In a first step the average over all dipoles for each frequency band was correlated with the tinnitus questionnaire. As this analysis yielded significant associations that were specific and consistent in direction for the alpha and delta band, values from each region were correlated with the scales of the questionnaire in order to help interpret effects gained in the first step.

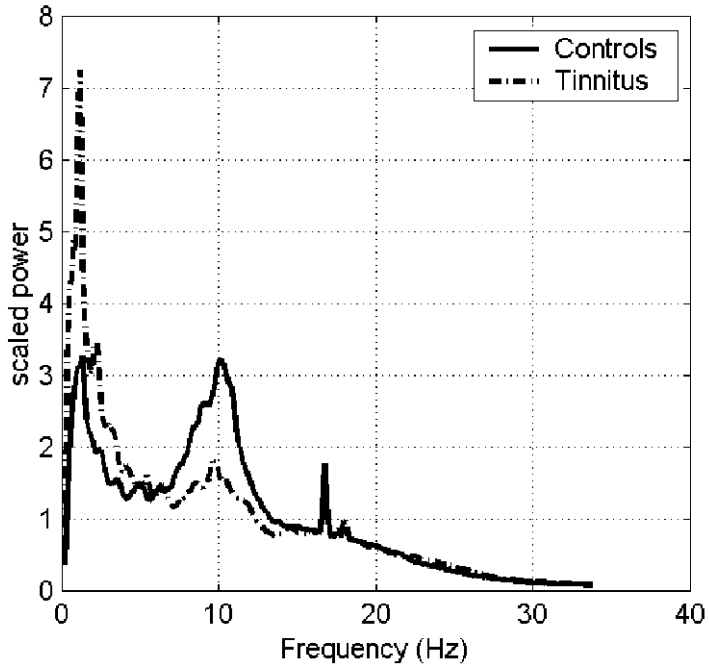

Figure 3-1: Power spectrum averaged over all sensors show a reduced alpha peak in tinnitus sufferers and an enhancement for delta. (The sharp peak centred at 16 2/3 Hz represents technical noise resulting from the 1km distant railway system).

3.1.3 Results

Spontaneous activity pattern: An explorative analysis of the frequency spectrum of the recorded magnetic fields revealed characteristic changes in tinnitus sufferers from the usual pattern seen in normal hearing controls (see Figure 3-1). The alpha peak, which can be seen in the control group is strongly reduced in the tinnitus group. In contrast, the power in the delta frequency band is considerably enhanced. This pattern is spatially resolved by the results obtained by the minimum norm solution which attributes both, the alpha attenuation and the delta enhancement mostly to the

temporal regions (Figure 3-2). It is clearly visible that the effect is considerably stronger concerning the alpha reduction as compared to the delta enhancement. A repeated measures ANOVA reveals a significant group by frequency band interaction ($F_{2,62} = 4.47$, $p < .03$). Differences between the values of each frequency range (alpha vs. theta, alpha vs. delta, theta vs. delta) were calculated for each individual and the results were subjected to a between subjects ANOVA. This analysis shows that the control group exhibits a significantly higher alpha than theta power ($F_{1,31} = 5.84$, $p < .03$), whereas the tinnitus group has stronger delta activity as compared to alpha ($F_{1,31} = 5.11$, $p < .03$). No differences were found between the groups when theta was contrasted with delta ($F_{1,31} < 1$). These results statistically confirm the impression of a reduced alpha and enhanced delta peak in the tinnitus group gained from Figure 3-1 and 3-2. This interaction pattern was statistically significant for all regions with the exception of right frontal and anterior parietal regions of both hemispheres. The foci of altered spontaneous activity pattern in the tinnitus group are clearly found bilaterally in temporal areas ($F_{1,31} > 5$, $p < .01$), which taken from Figure 3-2.

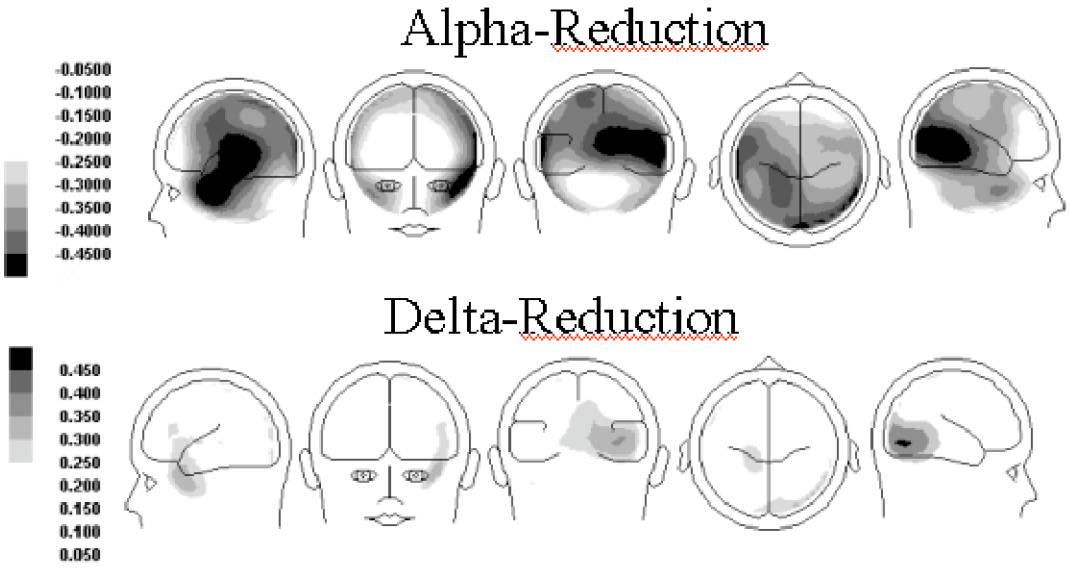

Figure 3-2: Difference maps between tinnitus sufferers and controls for alpha (top panel) and delta (bottom panel). The result suggests that areas for which alpha reduction and delta enhancements are found partly overlap. Overall the effect for the alpha band is considerably stronger.

Association of spontaneous activity with tinnitus-related distress: For both alpha and delta power, significant correlations were found with the Tinnitus Questionnaire, ranging between .5 and .7 (see Table 1). The distribution of correlation coefficients for the total score of the Tinnitus Questionnaire is shown in Figure 3-3.

To exclude the possibility that the high associations are a consequence of potentially different subgroups – i.e., one subgroup high in delta and another one with low alpha – a frequency index was calculated [(delta-alpha) / (delta+alpha)] for each individual. The correlation of this score with the Tinnitus Questionnaire is displayed in the bottom panel of Figure 3-3. Strongest associations with this measure are found in right temporal and left frontal regions. Looking at the frequency bands separately however, this pattern is overall more pronounced for the alpha reduction than the delta enhancement (see Table 1). Also, correlation coefficients are generally stronger in the case of the alpha reduction. Results of the theta band did not correlate with any of the questionnaire data. As only a single significant correlation could be observed with the sleeping problems subscale, the explanation that the results simply reflect sleeping disturbances can be discarded.

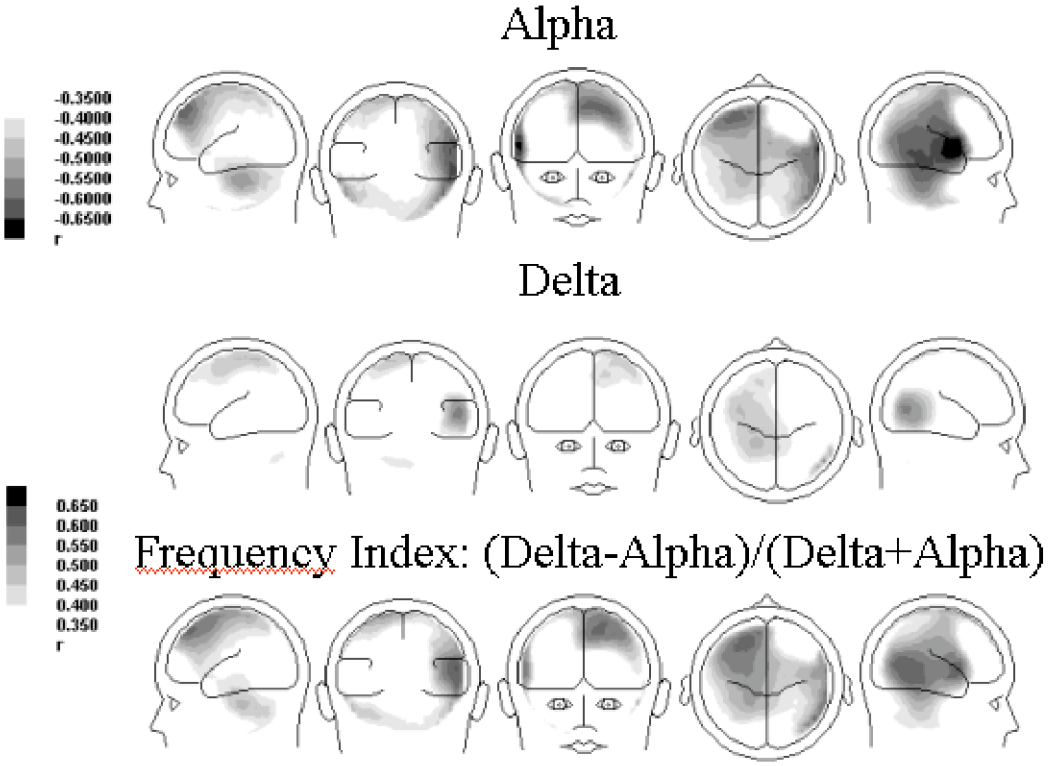

Figure 3-3: Correlation map between alpha (top panel), delta (middle panel) and tinnitus related distress (total score). Since previous analyses (see Figure 3-2), implicated corresponding areas for the effects found for alpha and delta, tinnitus related distress was additionally correlated with a frequency index ([delta-alpha]/[delta+alpha]; bottom panel). Effects are largest for right temporal and left frontal sources.

Table 1: Correlation coefficients between power in frequency bands and scores on the Tinnitus Questionnaire. Statistically significant correlations are highlighted in grey. Note that significance level has not been corrected (see Methods for rationale).
significance assessed with t-test (df = 15)
Gerneral: *italic*: $p < .05$;
italic & **bold**: $p < .01$;

		LO	RO	LPP	RPP	LPA	RPA	LT	RT	LF	RF	LPF	RPF	**Total**
Correlations with TF	**Alpha**	-.39	-.41	-.41	-.42	-.48	*-.53*	-.44	***-.65***	*-.60*	-.27	-.30	-.32	*-.51*
		-.44	-.43	-.41	-.40	-.48	*-.49*	*-.50*	***-.64***	***-.62***	-.30	-.40	-.36	*-.54*
		-.44	*-.49*	*-.50*	*-.51*	*-.54*	***-.62***	*-.53*	***-.70***	*-.59*	-.35	-.36	-.36	*-.58*
		-.41	-.47	-.48	*-.51*	-.47	*-.60*	-.48	***-.68***	*-.58*	-.33	-.27	-.37	*-.55*
		-.32	-.34	-.30	-.29	-.34	-.42	-.34	*-.54*	-.45	-.20	-.24	-.25	-.41
	Theta	.10	-.01	.21	.24	.06	.21	.04	.41	.06	-.10	.16	.09	.24
		.02	-.15	.10	.08	-.00	.10	.03	.32	.00	-.04	.14	.06	.15
		.21	.16	.34	.39	.21	.30	.19	.48	.18	.01	.32	.22	.39
		.18	.06	.29	.33	.05	.28	.00	.36	.01	-.10	.11	.11	.25
		.10	.05	.21	.20	-.03	.17	.10	.44	-.00	-.11	.05	-.01	.18
	Delta	.42	.46	.44	.45	*.53*	.45	.43	*.50*	.48	.35	.30	.34	.48
		.51	*.53*	.44	.47	*.49*	.40	.41	*.50*	.45	.32	.31	.38	*.49*
		.40	.41	.46	.47	*.49*	.45	.41	.47	.36	.28	.22	.24	.43
		.40	.46	*.52*	*.52*	*.58*	*.52*	*.52*	*.54*	*.52*	.44	.34	.37	*.52*
		.34	.40	.30	.27	.40	.29	.33	.43	.38	.28	.27	.30	.39

3.1.4. Discussion

The data presented here resemble the first group study on tinnitus in humans (there have been reports on single cases; see Jeanmonod et al., 1996; Llinas et al., 1999) to show marked alterations in spontaneous cortical activity. Notable group differences are a bilateral enhancement in delta and an accompanying reduction in alpha power over temporal areas (extending into posterior regions in the right hemisphere). The results are in agreement with ideas put forward by Jeanmonod and Llinas (Jeanmonod et al., 1996; Llinas et al., 1999), who stated that positive symptoms are a consequence of a hyperpolarization of thalamic neurons following deafferentation, leading to spike bursts around 4 Hz. The fact that regions with an increase in slow-wave activity are also the regions of decreased alpha activity resemble results found during slow wave sleep (Benoit et al., 2000) and support the idea that the changes in spontaneous brain activity might be mediated by sensory deprivation, i.e., partial hearing loss in this case. Additional evidence is provided by the observation that delta enhancement and alpha reduction were strongly correlated with tinnitus related distress variables with a focus on the right temporal and also left frontal cortex. Even though the data conforms generally with predictions based on Jeanmonod and Llinas ideas, it has to be stated clearly that that abnormal thalamocortical interaction can only be a piece in the puzzle of a 'neuronal tinnitus network'. The reason for this is that medial thalatomy as performed by Jeanmonod et al. (1996) on six patients brought relief for only about half of the patients, with a full abolishment of the symptomatic in one.

The association with distress suggests that the right temporal and left frontal cortex might be involved in a tinnitus related cortical network, in which the temporal region is associated more with the perceptual and the left frontal region more with the affective distress and motivational attention of tinnitus. Concerning the stronger effect for the right temporal area rather than the left, one has to consider the higher frequency of left sided tinnitus in our study. Thus it can not be excluded that this asymmetry would vanish when more subjects with right sided tinnitus would have been found. However, the fact that tinnitus is generally more common for the left ear (Lenarz, 1992) certainly opens up possibilities to speculate about potential asymmetries, either on a peripheral or central level that could account for this. The underlying physiological reasons for this asymmetry on an epidemiological level has

not been a matter of research so far. Left frontal activation has been linked with positive and right frontal activation with negative affect (Davidson & Irwin, 1999; Davidson, Pizzagalli, Nitschke, & Putnam, 2002; Wheeler, Davidson, & Tomarken, 1993). In the context of this framework, enhanced alpha (indicating a hypoactivation) in the left frontal cortex should be indicative of depression (a positive association between left prefrontal alpha activity and depression scores was also recently demonstrated in our working group; Slapin et al., unpublished data). The present alpha-distress association however is negative, thus implicating that the results obtained can not be explained by an effect of higher depressive mood in tinnitus sufferers. This simple explanation would also not fit with recent data from our workgroup (Wienbruch, Moratti, Elbert, Vogel, Fehr, Kissler, Schiller, & Rockstroh, 2003), which demonstrated a negative association between left frontal delta (measured via dipole density) and depression. In future studies, the elucidation of the function of the (left) frontal area is relevant, as it may point us to the role of top-down influences, that presumably play a role in the perception and perhaps even generation of this auditory sentiment.

3.2. Study 2: Auditory Mismatch response

3.2.1. Aim

This EEG study was motivated particularly by works on reorganization of the auditory cortical tonotopic map following a hearing damage, described in sections 1.2.2. and 1.2.3.. As literature not only reports of general enhancements of neuronal responses to lesion-edge receptors (e.g., Dietrich et al., 2001) but also of concrete ameliorations in frequency-discrimination performance (McDermott, Lech, Kornblum, & Irvine, 1998; Thai-Van et al., 2003; Thai-Van et al., 2002) interpreted as a consequence of injury-induced plasticity, the auditory mismatch response was regarded as a potentially useful tool to investigate abnormal neuronal responses in tinnitus sufferers. The auditory mismatch response is an evoked response of the brain that is elicited when a rare (< 15 %) acoustic stimulus deviates (i.e., the deviant) in a certain feature (frequency, loudness, duration, etc.) from a frequently presented standard. It reflects the capacity of the central nervous system to detect changes to a trace of a standard in sensory memory (Näätänen & Alho, 1997; Näätänen & Escera, 2000). Due to the work on tonotopic map reorganization and the cited psychoacoustical studies an enhanced auditory mismatch response was expected. Another goal consisted in studying if different neuronal generators are involved in the processing of the auditory stimuli as compared to normal hearing controls. Effects seen on a group comparison level were related with tinnitus related distress which was assessed using a standard German questionnaire (Tinnitus Fragebogen). Furthermore, as hearing loss is assumed to be fundamental for the development of tinnitus, questionnaire data and relevant neurophysiological variables were correlated with parameters of hearing loss (depth and steepness / slope). In this study, a strong correlation of paradox lesion-edge effects with the emotional distress caused by tinnitus could be observed, pointing to the potential role of top-down modulation of auditory cortex functioning.

3.2.2 Methods

Participants: The tinnitus and control group were matched by sex (13 males, 2 females) and age (tinnitus: mean age (range) = 47.27 (24-65); controls: 46.67 (24-63). All members of the tinnitus group experienced either unilateral or bilateral moderate to severe high-frequency hearing loss, and a tonal tinnitus. Four tinnitus sufferers

could not recall the exact elicitors for their condition, nine of them reported to have had a long professional exposure to noise and two mentioned noise trauma as the origin of their disorder. No participant was aware of the hearing-loss (not uncommon in cases of high-frequency hearing loss) prior to the experience of tinnitus.

Stimuli and Procedure: Prior to the experiment written informed consent was obtained and the Tinnitus Fragebogen completed. For the mismatch experiment the patient was placed in a sound-attenuated chamber. All stimuli were sinusoidal tones of 70 ms duration (10 ms rise and fall). A mismatch procedure using one standard (85% probability) and three deviants (3 x 5% probability) was employed. Two conditions were defined based on visual inspection of a high-resolution audiogram of each tinnitus subject: In the *Lesion-Edge (LE)* condition, the standard was defined as the lowest frequency before hearing threshold started to deteriorate (i.e., audiometrically normal frequency prior to the downward slope in the audiogram). Stimuli were presented monaurally via headphones (HD520II, Sennheiser). Stimulation was presented to the ear in which the participant localized the tinnitus (ipsilateral tinnitus) or the one in which the tinnitus was perceived to be stronger (bilateral tinnitus with a dominant side). For the *Control (CO)* condition, the standard was set one octave below the LE frequency. Deviants were chosen to be 1, 2 and 4% lower in frequency than the standard in each condition. For each tinnitus patient, a control subject was matched by age and gender. The respective stimulus was presented to each patient's control subject. Three runs of each condition were alternated, with the starting condition balanced across subjects. Overall, 3701 stimuli were presented per condition with an ITI of 700 ms. During this time, participants were instructed to read a book of their choice. Prior to the actual experiment, the stimuli were matched in loudness to a 1000 Hz sinusoidal tone set at 65 dB SPL.

Data acquisition and analysis: Neuroelectric signals were recorded and corrected for eye-artefacts as described in section 2.1.. A time-window of 30 ms around the peak in the global field power of each individual was selected and two symmetric (ipsilateral and contralateral to stimulation) regional sources were fitted to the data (average reference). Source localizations obtained from this analysis were used to obtain the source waveforms of each stimulus type (standard and deviants) for each condition (LE and CO) separately. Orientations of the dipoles were adjusted to the maximum value of the global field power (peak of N1 in all cases). To minimize

the possibility of certain subjects with extreme values and effects, source waveforms were standardized using the vector scaling method proposed by McCarthy and Wood (1985). The source-related mismatch activity was defined as the difference in activity between the respective deviant and its standard. To test whether the effect found on the posterior-anterior axis (localizations further anterior in tinnitus and high correlation with distress scores) were a consequence of enhanced frontal activity the MNE (Hämäläinen et al., 1993; Hauk et al., 2002) was employed (computations done with BESA2000; 162 dipoles distributed on a sphere). Prior to MNE, the grand average of each individual was projected onto a common standard electrode configuration. Following this, the overall grand average of the normal-hearing control *group* was subtracted from each tinnitus *individual*, i.e. the control group served as a kind of baseline. These data were then used for the MNE analysis, and the resulting source waveforms were analogously scaled as described above. The results were subsequently averaged between 90-135 ms (i.e., the N1 time-window), thus resulting in a vector with 162 values (one per dipole) for each tinnitus individual. Since it was assumed that the source localizations observed using the symmetrical dipoles could have been the consequence of activity stemming mainly from frontal and temporal sources, the mean scaled activity values of clusters of dipoles in these regions were divided (i.e., frontal / temporal) for both hemispheres. This index gives some information on how much more active frontal regions were as compared to temporal. Based on the notion that enhanced frontal activity would draw the localization of the fitted dipole further anterior, we expected a linear relationship.

3.2.3 Results

The 4 % deviant produces the largest amplitude differences relative to the standard ($F_{2,56} = 22.46$, $p < .001$). For the LE condition, Figure 3-4 depicts a striking abnormality in the tinnitus group that appears in the 90-135 ms time-window. Whereas the control group shows a marked deviance-dependency (1% < 2% < 4%), this is not the case for the tinnitus group. The amplitude following the 1% deviant is enhanced, while the amplitude of the 2% deviant appears to be reduced. For the LE condition, but not for the control frequency, an interaction between Group and Deviant ($F_{2,56} = 6.73$, $p < .003$) was found. Post-hoc analyses of the within factor Deviance using Tukey-Kramer HSD show a significant difference in the tinnitus

group between 2% and 4% (mean difference: -.17, critical difference: .12); statistical significance was attained in the control group for the difference between 1% and 4% (mean difference: -.34, critical difference: .17), 2% and 4% (mean difference: -.19). An unpaired t-test yields a significant difference between tinnitus and controls for the 1% deviant (mean difference: .14; t_{28} = 2.71, p < .02) and 2% deviant (mean difference: -.11; t_{28} = -2.27, p < .04), but not for the 4% deviant (mean difference: -.13; t_{28} = -1.54, p > .1).

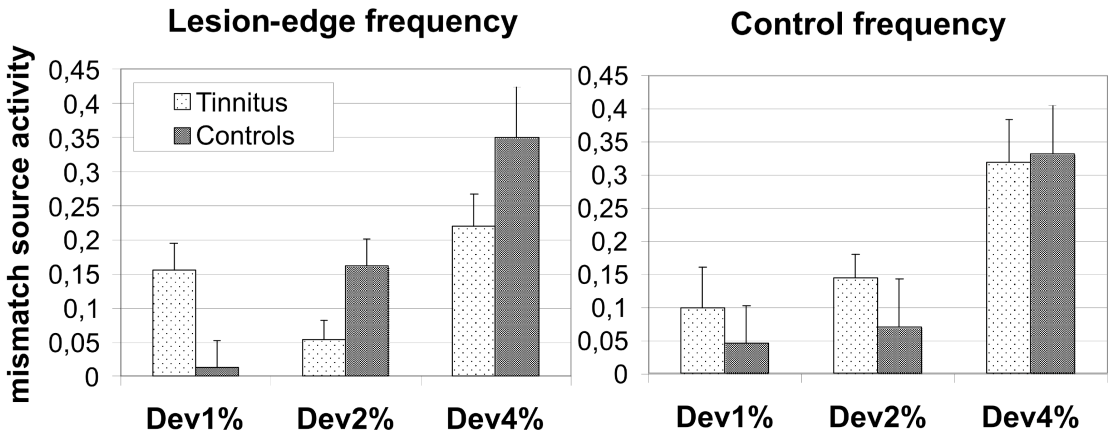

Figure 3-4: Mismatch related source activity (scaled). Responses to standards were subtracted from those to deviants whereby positive values indicate a greater neuronal response following the deviant. Normalized amplitudes (source strength) of the mismatch negativity are displayed for the time segment from 90-135ms.

Source localization of the N1 showed significant differences between groups in the inferior-superior (tinnitus: *Mean* = 65.17 mm / *Standard Error* = 2.92, controls: 52.55 / 4.85; $F_{1,28}$ = 4.97, p < .04) and posterior-anterior direction (tinnitus: 4.38 / 3.89, controls: -5.51 / 1.69; $F_{1,28}$ = 5.44, p < .03). Groups did not differ in the medial lateral direction (tinnitus: 43.88/ 2.08, controls: 45.17 / 1.58; $F_{1,28}$ = 0.23).

Taken together, differences were found for the location of cortical response around 100 ms and for the 1% and 2% mismatch response in the 90-135 ms period of the LE condition. These variables were used to assess associations with tinnitus related psychological distress (measured using a standard German questionnaire; Goebel & Hiller, 1998) by calculating simple linear regressions. As one subject (mti011) scored zero points on this subscale, thus representing an extreme, this case was excluded for the main calculation with neurophysiological variables (results with this case included are however also reported). The analysis reveals that source localizations in the posterior-anterior direction and the mismatch related source

strength to the 2% deviant are strongly correlated with distress variables, i.e.: higher distress scores are associated with more anterior sources (Figure 3-5a; $r = .76$, $F_{1,13} = 16.60$, $p < .002$; with mti011: $r = .53$, $F_{1,14} = 5.01$, $p < .04$) and *stronger* mismatch activation for the 2% deviant ($r = .74$, $F_{1,13} = 14.33$, $p < .003$; with mti011: $r = .64$, $F_{1,14} = 9.72$, $p < .009$). The association between the distress scores and source localization in inferior-superior direction were not comparable in magnitude (range: $.37 < r < .47$) and also failed to reach statistical significance.

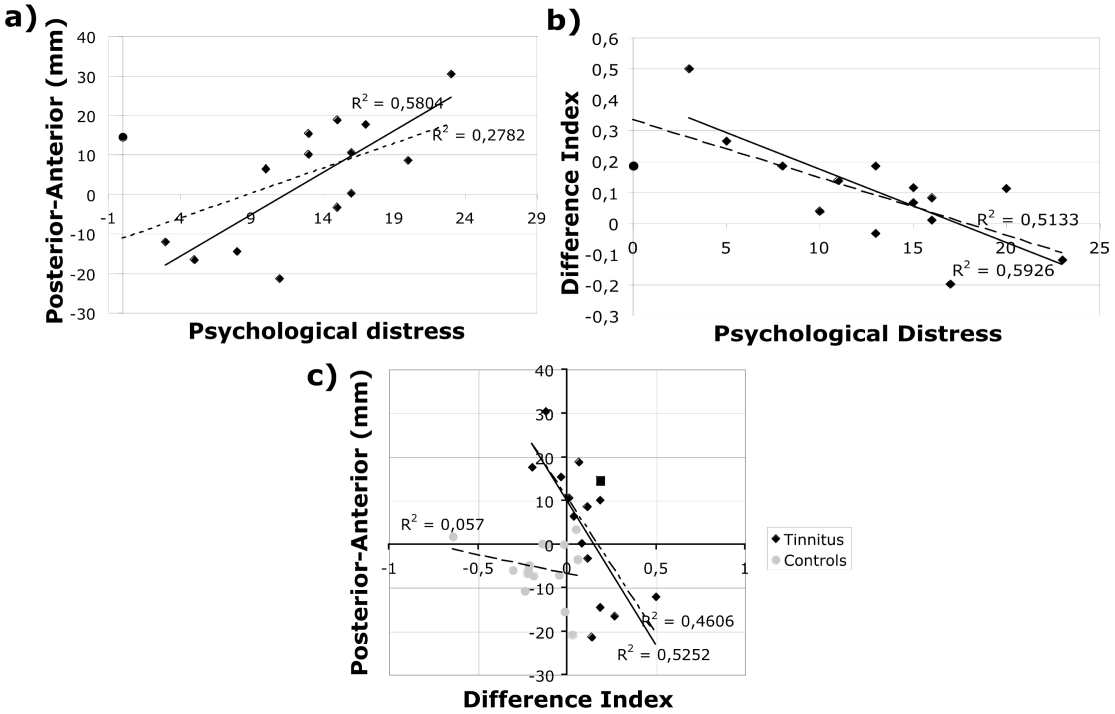

Figure 3-5: Scatterplot of neurophysiological variables. Associations between Psychological Distress (i.e., Emotional + Cognitive Distress; measured with the Tinnitus Questionnaire) and a) source localization in the anterior-posterior direction and b) the difference between the mismatch response of the LE condition to the 1% and 2% deviant in the early time window (Difference Index; negative values indicating a more normal mismatch pattern, i.e. higher responses for the 2%-Deviant). c) The correlation between the two neurophysiological variables. Note that they are highly correlated for tinnitus but not for control subjects, and that both groups are almost perfectly separated by these two variables. Concerning all scatterplots: straight line corresponds to fit without mti011 (circle in a) and b), square in c)), broken line to the fit including this subject (see text for details).

Since an enhanced activation for the 1% deviant in the LE condition and a reduced activity for the 2% deviant seemed to be at the core of the abnormal response in tinnitus, it was tested whether the difference between mismatch-related source activity for the 1% and 2% deviant would also show significant relationships with distress variables. This was indeed the case, i.e. the more negative this index (and therefore, the more "normal") the stronger the distress (see Figure 3-5b; $r = -.77$, $F_{1,13}$

= 17.43, $p < .002$; with mti011: $r = -.72$, $F_{1,14} = 13.68$, $p < .003$). In Figure 3-5c it can be seen that the mismatch and source localization related variables are well correlated for the tinnitus group ($r = .72$, $F_{1,13} = 13.24$, $p < .004$; with mti011: $r = -.69$, $F_{1,14} = 11.09$, $p < .006$) but not for the control group ($r = .23$, *n.s.*). In the tinnitus group, the more normal the mismatch in the early time window of the LE condition (i.e., the more negative the difference index), the more anterior the focus of neuronal activity.

Also the possibility was examined that the anterior shift of source localization and its considerable variability may be caused by an enhanced frontal activation, relative to the expected temporal activation. This was suggested by the difference in the source distribution between controls and tinnitus subjects using MNE (see Methods), as shown in Figure 3-6 for the 112 ms latency (maximum difference; upper panel). The results (see Figure 3-6 lower panel) suggest that a linear relationship exists between the two variables for the majority of cases. However, there is a subset of four patients with the most posterior source localizations. These patients exhibit strong frontal activation, thus causing the overall association to be quadratic. This relationship is statistically significant for the *right* hemisphere only ($F_{2,14} = 6.32$, $p <$ 02). Interestingly, these four patients are exactly the ones who reported the lowest distress values (see Figure 3-5a).

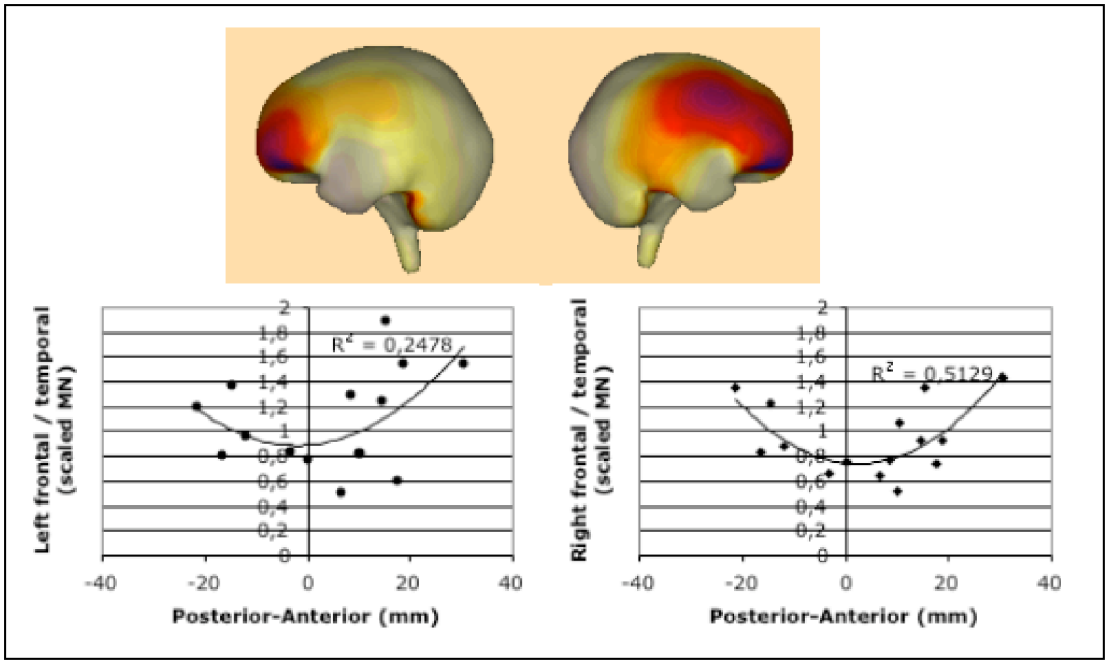

Figure 3-6: Upper panel: The minimum-norm solution for the difference between the Grand Means of the tinnitus and the control groups indicates a greater prefrontal activity (dark shading) in tinnitus patients following auditory stimulation. The darkest shading corresponds to 100% of the maximum activation (the lighter, the stronger the

decrease relative to the maximum). This exploratory analysis was the basis for the post-hoc notion that the source localization in the anterior-posterior direction may be associated with prefrontal activity. Lower Panel: Correlating relative frontal activity (as compared to temporal sources) of each individual with the source location of the regional source results in a quadratic trend that is more pronounced for the right hemisphere.

Finally, concerning the role of the hearing loss parameters (depth and steepness), steepness appears to be relevant. This audiometric variable is significantly correlated with tinnitus related distress (see Figure 3-7c; r = -.64, $F_{1,13}$ = 8.64, p < .02; with mti011: r = -.44, $F_{1,14}$ = 3.24, p < .09). The direction of the correlation implies that *steeper* hearing losses are related to *lower* distress values. Furthermore steepness showed a significant association with the difference index of the mismatch at LE (see Figure 3-7b; r = .68, $F_{1,13}$ = 10.49, p < .007; with mti011: r = .64, $F_{1,14}$ = 9.18, p < .01), while being uncorrelated (r = .19) with the analog difference index for CO. Although the correlation with source localization in the posterior-anterior direction was also significant (see Figure 3-7a; r = -.54, $F_{1,13}$ = 4.97, p < .05; with mti011: r = -.56, $F_{1,14}$ = 5.80, p < .03), this effect seemed to be driven by the four subjects with the steepest hearing losses. All associations with depth of hearing loss were not significant (all Fs < 2). Steepness and depth of hearing loss were uncorrelated (r = -.16, $F_{1,14}$ = 0.31, p < .59).

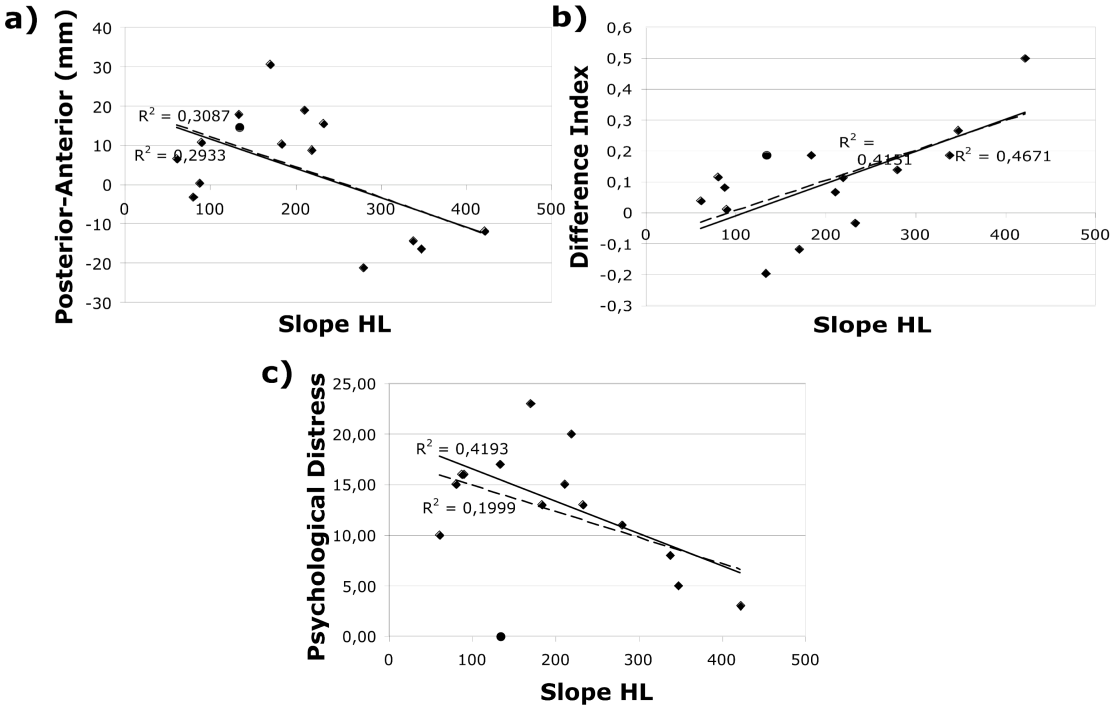

Figure 3-7: Scatterplot of steepness of hearing loss (larger values corresponding to steeper slopes; see Methods for details). a) The correlation of slope and source localization in posterior-anterior direction. Though being statistically significant the

association is not very convincing and seems to be largely driven by four subjects with the steepest hearing losses. b) Association of steepness of hearing loss with the difference index of the mismatch (see also Figure 2b) shows a strong positive correlation. c) Hearing loss slope is negatively associated with tinnitus related distress. Concerning all scatterplots: straight line corresponds to fit without mti011 (as circle), broken line to the fit including this subject (see text for details).

3.2.4. Discussion

Overall, the notion of a general enhancement of the auditory mismatch response at lesion edge frequencies could not be confirmed. This is not necessarily a contradiction to Dietrich et al. (2001), as our study investigated the difference between two responses. The response pattern is generally more complex incorporating enhanced (1% deviant) *and* reduced (2% deviant) neuronal activity. It is noteworthy to emphasize that there was no difference to the control group in the control condition. The reason for this paradoxical result can not be derived from the present study. However it is difficult to argument with peripheral mechanisms, as the LE frequency was chosen by visual inspection from the audiogram: this measure does not offer detailed information about the type and extent of damage to receptors in the inner ear. It is striking that the time window of the effect is almost identical as the one reported by Jacobson et al. (1996) on the enhanced negative difference wave (100-140 ms) in tinnitus, indicating an increased attention in this group. It is also conceivable for the present study that (involuntary) attentional mechanisms were activated differently in the tinnitus group as compared to the control group; mechanisms, that might not be elicited in a classic N1 design as employed by Dietrich et al. (2001). Additionally, the relationship between the mismatch response and tinnitus related distress indicates that a *more normal* mismatch pattern is associated with enhanced distress scores. Subjects with the most abnormal mismatch patterns were those to exhibit lowest distress values. However, the more normal mismatch pattern in distressed subjects is not comparable with the pattern seen in normal hearing controls, as there is a considerable shift of the focus of neuronal activation in an anterior direction. Based on this finding, I hypothesize that a damage to hearing receptors of the inner-ear trigger reorganization processes leading to an abnormal mismatch pattern as found in the patients with low distress. With an increase of the distress level however, frontal brain regions involved in emotional and attentional regulation modulate this mismatch response. Prefrontal activity has been shown to be involved

in processing auditory information previously (Romanski & Goldman-Rakic, 2002), also for the auditory mismatch response (Alho, Woods, Algazi, Knight, & Näätänen, 1994; Jemel, Achenbach, Muller, Ropcke, & Oades, 2002). Our data indicate a possible involvement of especially right prefrontal areas. Yet, caution has to be taken due to the low spatial resolution offered by EEG. A further aspect to consider is that a feature of the audiogram, namely slope of the hearing loss, is associated to both psychological distress and the mismatch difference index. Together with the specifity of the mismatch effects for the LE, this finding underlines the importance of seeing tinnitus *in conjunction* with a damage of the hearing system and not as two independent phenomena. Steepness of the hearing loss is positively associated with the abnormality of the mismatch response at LE as reflected in the difference index. Both variables are negatively related with tinnitus related distress, i.e. high scores are correlated with a more normal mismatch pattern and flatter hearing loss slopes. In summary, these results imply that a too simple reorganization logic (i.e., peripheral damage leading to expanded edge frequency representation and this resulting in tinnitus) that also underlay the present study is perhaps too naive, at least in the context of tinnitus. A better understanding of how auditory representational areas reorganize following a damage to receptors and how these processes act *in concert* with top-down mechanisms seems to be of big importance for a better understanding of this auditory phantom perception in general.

3.3. Study 3: Indicators of altered tonotpic representation

3.3.1. Aim

In the first MEG attempt to investigate tinnitus in a similar manner as previously phantom pain (Flor et al., 1995) by Mühlnickel et al. (1998), deviations of the 'tinnitus frequency' from expected location on the tonotopic map could be shown to be correlated with perceived tinnitus strength. I discussed the several shortcomings of this approach in section 1.2.3., among other things the exclusion of subjects with audiometric hearing loss which makes a comparison of the results with those on phantom limb pain difficult. A study ensuring better comparability should incorporate at least a moderate receptor damage, which could be operationalized e.g. by hearing losses exceeding 25 dB HL. Also, a phantom-pain-like study of tinnitus would focus on altered representational features of the audiometric (lesion) edge frequency, rather than the 'tinnitus frequency' which usually is located somewhere in the hearing loss: this frequency (region) would correspond better to the face region neighbouring the (amputated) arm region on the somatotopic map. A study with a design of the kind just described was done by Dietrich et al. (2001) in which the authors could show *selectively* enhanced neuronal activation for lesion-edge frequencies. This effect was however not correlated with the amount of perceived tinnitus distress. The approach in this study (14 tinnitus, 11 normal hearing controls) was very similiar: lesion-edge and control frequency were determined in a similar manner as in the Mismatch study (section 3.2.2.) and pure tones were presented blockwise unilaterally for each ear (i.e., overall 4 blocks). After several studies on tonotopic representation in our laboratory (Weisz et al., 2004a, 2004b; Wienbruch et al., unpublished data) which demonstrate tonotopy on a group level, but also the considerable interindividual variability I opted for a very liberal operationalization of tonotopy: the main criteria was that source locations for two carrier frequencies should not fall onto the same spot for the main tonotopic gradients (posterior-anterior, medial-lateral). That means, that tonotopy was quantified as distance (D; in mm) from a regression line with zero intercept and a slope of one in a scatterplot with spatial locations for lesion-edge and control frequencies as x- or y-axis respectively. Besides of tonotopic representation a look was taken at source strength, i.e. a replication attempt of the results from Dietrich et al., and the latency of the neuronal response.

3.3.2. Methods

Participants: Fourteen tinnitus (M = 49.64 years, SE = 3.30) and 11 normal hearing control male subjects (M = 43.18 years, SE = 4.37) participated in this study. Age between the groups did not differ significantly (t_{23} = -1.20, p = .24). All tinnitus subjects exhibited a moderate to severe high-frequency hearing loss. Written informed consent was obtained from each individual. Additionally, tinnitus subjects filled out the Tinnitus Fragebogen (Goebel & Hiller, 1998).

Procedure: Pure tones (50 dB SL; 300 ms; 10 ms rise / fall) were presented monaurally via plastic tubes with an ITI varying between 2-2.5 s. For the tinnitus participants two frequencies were chosen according to the audiogram: 1) the lesion-edge frequency (LE) resembled the audiometrically normal edge frequency of the hearing loss slope. 2) A control frequency (CO) was set at an octave below LE. Control subjects were assigned the same stimulus set of a patient, whom he resembled most age-wise. Blocks of conditions (300 tones per condition) were presented for both ears separately in a random order, thus resulting in overall four runs. Hearing loss slope was defined as described in section 2.4..

Data Acquisition: Recording of neuromagnetic data and eye-artefact correction were perfomed as described in section 2.1..

Data Analysis: For each condition 300 epochs of 700 ms length (200 ms pre-stimulus baseline) were extracted from the eye-artefact corrected continuous data. Epochs still contaminated by artefacts were rejected by visual inspection using the artefact scanning tool of BESA. The remaing epochs were averaged and bandpass filtered (1-20 Hz; Butterworth characteristic 2nd order). The goal of source analysis was twofold: 1) to detect possible spatial deviations from normal tonotopic representation and 2) to determine the neuronal response strengths for each condition. Therefore a montage consisting of eigth regional sources was created (Figure 3-8), of which two were placed symmetrically bilaterally in the auditory cortex. The purpose of this measure was to ensure that the two sources of interest (i.e., the auditory ones) do not pick up too much activation of surrounding sources, i.e. the other six dipoles acted as a kind of spatial filter. To determine source localization (spherical volume conductor with single homogeneous shell) a time window of 30 ms around the peak of the most prominent response was used (N1m), and the two auditory sources were fitted simultaneously to the observed data under the only constraint for them to be

symmetric. For analysis of dipole strength fixed locations of the auditory dipoles were used for all subjects (see Figure 3-8) and the orientations adjusted individually for all conditions. Time windows for the components were set from 35 to 75 ms for the P1m, 75 to 150 ms for the N1m and 150 to 300 ms for the P2m. Since no substantial activation could be found for orientation 2, source strength is reported for orientation 1 only.

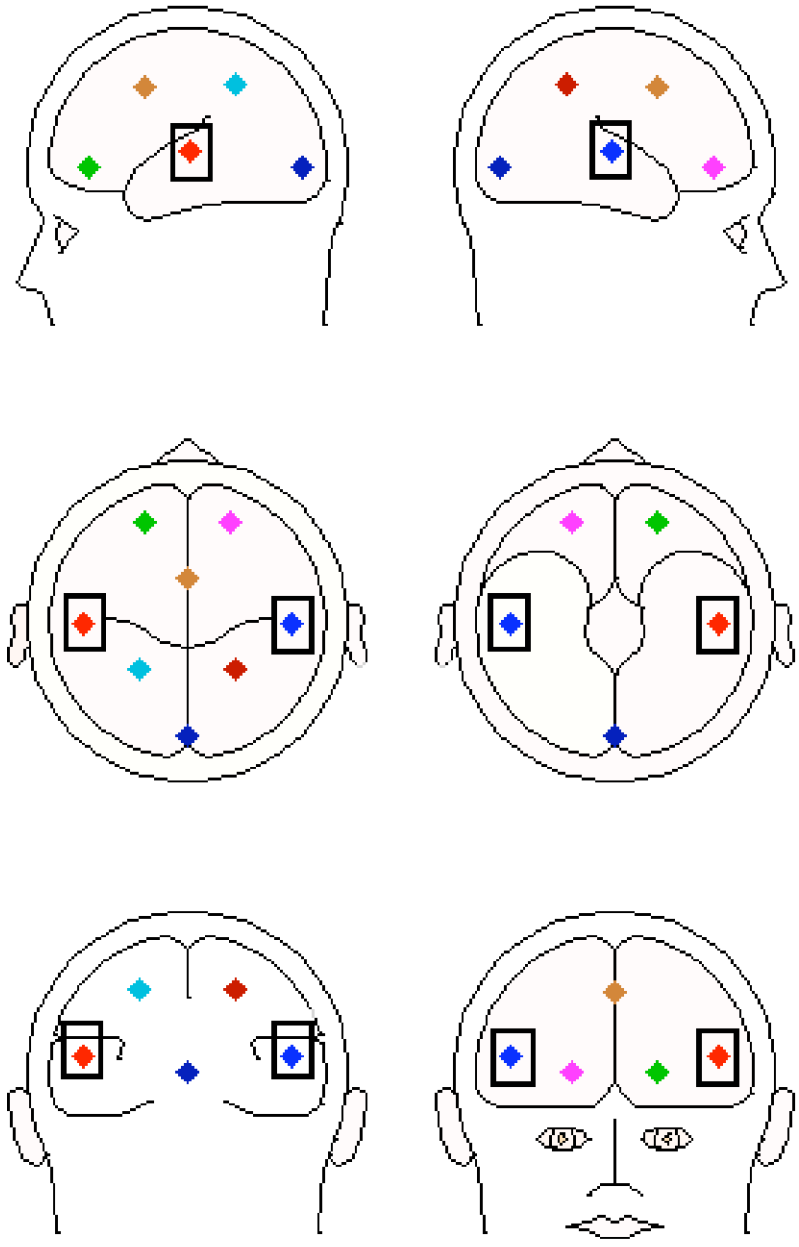

Figure 3-8: In order to minimize the influences of sources outside the auditory cortex, a source montage was constructed. The main sources of interest were bilaterally symmetrical in the auditory cortex. Dipole strength was investigated via a fixed montage, whereas for dipole location the two auditory sources were freely fit under the constraint for them to be symmetrical.

Operationalization of Tonotopy: As mentioned in the introduction, analysis of tonotpic representation is not a trivial undertaking (see Appendix A and B) and high subject variability can pose severe problems when trying to analyse differences between groups. Another source of unwanted variance comes from the fact that tested frequencies were adjusted individually according to the audiogram. Thus, for the present purposes a rather liberal operationalization of tonotopic representation was introduced: it was defined as distance (in mm) on one spatial gradient from a straight line with intercept 0 and slope 1 in a scatterplot in which the respective coordinate (x, y, or z) for LE represented the x-axis and the for CO on the y-axis. This line resembles the case in which localization for CO and LE were identical. Larger values indicate an increased distance between CO and LE and thus an expanded tonotopic representation.

The formula for distance was:

$$D = 0.5*\mathrm{sqrt}((x_{co}-x_{le})^2),$$

where D means distance in mm, x_{co} and x_{le} represent the spatial coordinate value for the posterior-anterior, medial-lateral or inferior-superior axis respectively.

Statistical Analysis: Since a pronounced P1m or P2m could not be identified in all cases only product-moment correlations with scores on the Tinnitus Questionnaire were calculated. Also not all subjects exhibited an ipsilateral N1m, thus repeated measures analysis (group x hemisphere x condition) was only done for the contralateral component. Outliers were excluded in cases where values exceeded more than 2.5 standard deviations of the group mean.

3.3.3. Results

Hearing-loss slope and tinnitus distress: A nonlinear negative association between slope and tinnitus related distress could be observed for the left ear but not for the right ear. It appears that this relation is especially present for left ear and bilateral tinnitus sufferers. To attain linearity the logarithm of the slope values was calculated (see Figure 3-9). A robust regression analysis using the *M*-method (Huber, 1964) was calculated for the left (left ear and bilateral tinnitus sufferers) and right ear (right ear and bilateral tinnitus sufferers). This statistics showed that only the relation for the left ear reached significance ($t_9 = 3.63$, $p < .003$; right ear: $t_9 = .39$, $p < .36$).

There were no significant associations between slope and neurophysiological variables, so they will not be mentioned further in the results part.

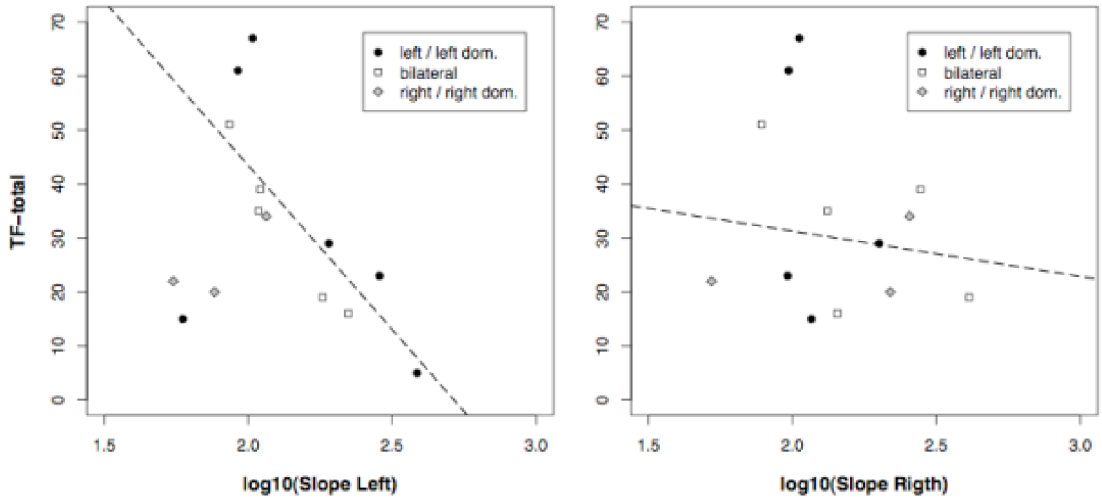

Figure 3-9: An nonlinear assocation between hearing loss slope and tinnitus related distress was specifically found for the left ear (left panel) independent of tinnitus laterality. A similar relationship could not be found for the right ear (right panel), even if only considering subjects with right-lateralized tinnitus.

P1m / P2m and tinnitus distress: Product-moment correlations between measures of the peak latency of the P1m and tinnitus related distress indicated significant negative relations for the left hemisphere after contralateral (r = -.68, t_{12} = -3.23, p < .008) and ipsilateral stimulation (r = -.62, t_{10} = -2.56, p < .03). A significant association could also be observed for the right hemisphere after ipsilateral stimulation (r = -.59, t_{12} = -2.56, p < .03). These correlations were specific for LE only (for CO all p > .08). Thus there seems to be a trend of faster processing of LE frequencies in tinnitus subjects with increasing distress. No relationship could be observed for the amplitude of the P1m. Also, all correlations with the P2m (latency and amplitude) were not significant (all p > .08).

N1m: Peak latency for the N1m was reached at 97.28 ms (SE = 2.93) in control and 101.12 ms (SE = 1.81) in tinnitus subjects. However, this group difference was not statistically significant (F(1,23) = 1.36, *n.s.*). CO tones peaked slightly slower than LE tones (100.32 ms, SE = 1.79; vs. 98.55 ms, SE = 1.58; $F_{1,23}$ = 6.60, p < .02). No significant associations with distress were found for this variable. Concerning amplitude one subject (case 14) was excluded from analysis due to excessive dipole moments. The source strengths are depicted separately for LE and CO in Figure 3-10. The average dipole moment lay around 13 nAm for both groups (controls: 13.35, *SE*

= 1.30; tinnitus: 13.82, *SE* = 1.43; $F_{1,22}$ = .06, *n.s.*) and were overall stronger for CO (14.15, *SE* = 1.04; LE: 13.06, *SE* = .93; $F_{1,22}$ = 4.69, $p < .05$). While values were approximately the same for LE (controls: 12.44 (left) / 13.18 (right), *SE* = 1.63 / 1.56; tinnitus: 12.70 / 13.85, *SE* = 1.64), they differed between the groups for CO depending on the hemisphere investigated (controls: 14.10 (left) / 13.35 (right), *SE* = 1.72 / 1.71; tinnitus: 12.43 / 16.30, *SE* = 2.42). Accordingly, a three-way interaction (group x hemisphere x condition) was obtained ($F_{1,22}$ = 11.00, $p < .004$). To resolve this interaction differences between the two conditions (CO - LE) were calculated for both groups and hemisphere separately (see Figure 3-11; positive values indicate stronger activation for CO). This analysis shows a reversed pattern for the two groups: normal hearing controls exhibit stronger activations for CO in the left hemisphere (1.66 (left) / .50 (right), *SE* = .65 / .65) whereas the tinnitus group has exactly the opposite pattern (i.e. stronger dipole moments for CO in the right hemisphere; -.26 (left) / 2.45 (right), *SE* = .77 / 1.04). A repeated measures ANOVA indicates a significant hemisphere effect for the tinnitus group ($F_{1,12}$ = 7.79, $p < .02$) and a strong trend for the control group ($F_{1,10}$ = 4.73, $p < .06$). There were no significant associations between tinnitus related distress and source strength.

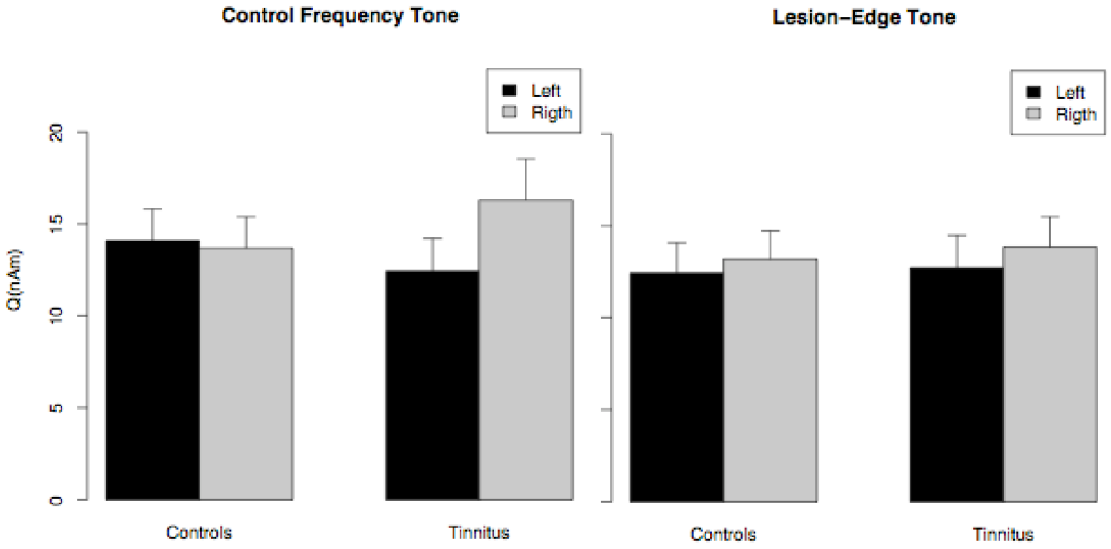

Figure 3-10: Source strength for CO (left panel) and LE (right panel). No differences between the two groups nor hemispheres become apparent in the LE condition. In the CO condition however, right hemispheric activity appeared to be enhanced in tinnitus subjects. This effect is further elucidated in Figure 3-11.

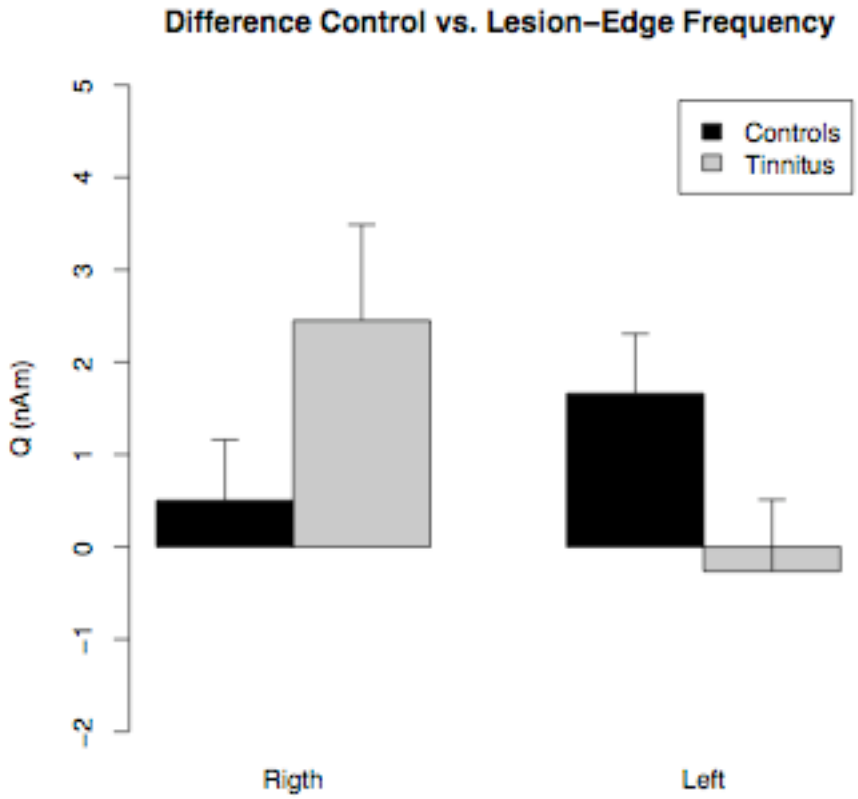

Figure 3-11: Difference between source strengths following the CO and LE tone (i.e. CO – LE) reveals a reversed pattern between the two groups. Whereas the normal hearing controls exhibit a trend towards stronger activations to CO tones (relative to LE) in the left hemisphere, tinnitus subjects show this stronger activation very pronounced in the right hemisphere.

Source location medial-lateral: Even though localization (case 1 excluded from analysis due to excessive values) for LE was approximately 2 mm more medial than CO and 4 mm more lateral in the left hemisphere than in the right, neither effect for condition nor hemisphere were statistically significant ($F_{1,22} \sim 1$, *n.s.*). However a significant group effect was obtained ($F_{1,22} = 7.18$, $p < .02$): independent of condition or hemisphere source locations for tinnitus sufferers (52.56 mm, $SE = 1.01$) were 4 mm more lateral than for the control group (48.55, $SE = 1.41$; see Figure 3-12). A positive correlation was observed for location of CO in the right hemisphere and tinnitus related distress ($r = .56$, $t_{11} = 2.26$, $p < .05$), i.e. higher distress values were related to more lateral sources. As mentioned in the methods section, tonotopic organization was operationalized as distance measure (D). Three cases (1, 5, 19; two tinnitus, one control) had excessive values and were excluded from further analysis. No significant group effect ($F_{1,22} = .18$, *n.s.*) was found for this variable, nor was there any association with tinnitus related distress.

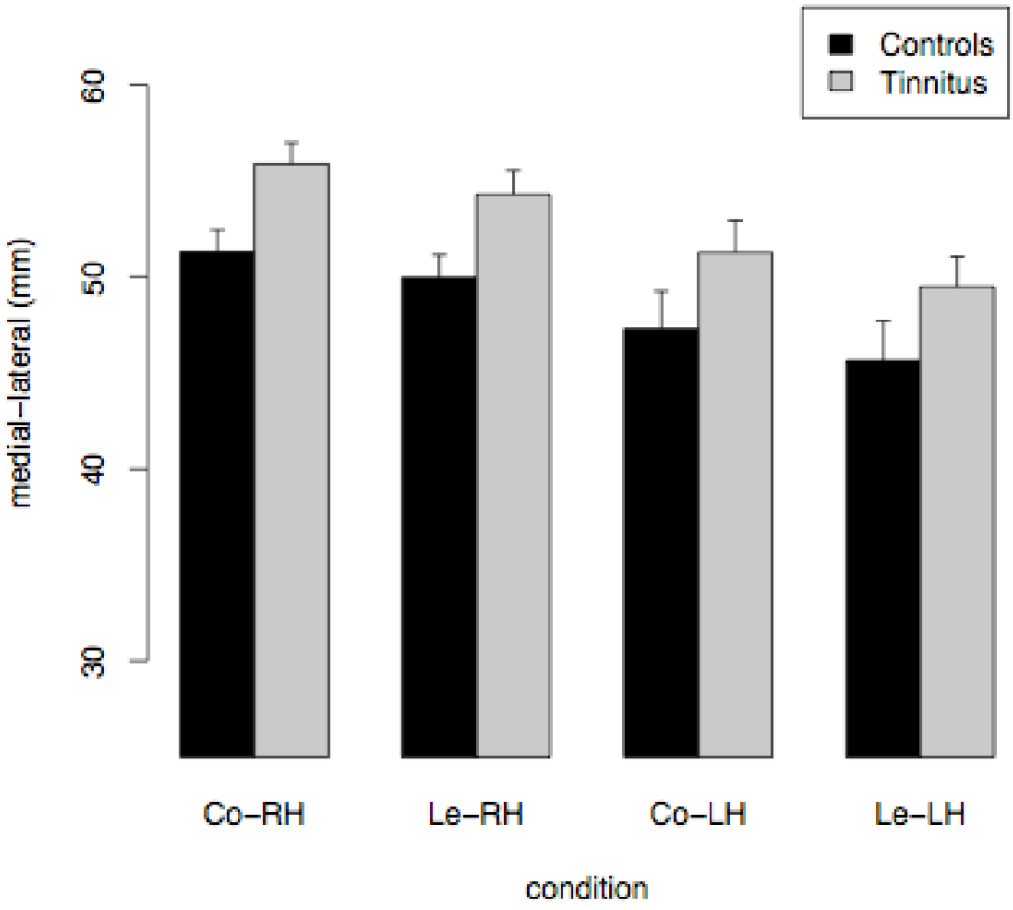

Figure 3-12: Independent of frequency or hemisphere, tinnitus sufferers have consistently more laterally localized sources than normal hearing controls.

Source location posterior-anterior: Average locations on the posterior-anterior axis are depicted in Figure 3-13. The frequently reported more anterior location of sources in the right hemisphere could be observed for the control group (~4 mm). In the tinnitus group location of LE appeared to be further posterior in the right hemisphere (14.11 mm, SE = 2.25; controls: 19.74, SE = 2.39), although no significant group x hemisphere x condition interaction could be observed ($F_{1,22}$ = .12, *n.s.*). However when analyzing D (case 1, 5, 16 not included due to excessive values; two tinnitus, one control), it becomes clear that differences exist between the two groups on this axis (see Figure 3-14). Similar to the effect for the N1m, a reversed pattern can be observed for the hemispheres (group x hemisphere: $F_{1,20}$ = 8.48, p < .009). Tinnitus subjects exhibited greater D values in the right hemisphere than

controls (1.76 mm, SE = .34; controls: .51, *SE* = .14) whereas it was smaller in the left hemisphere (1.02, *SE* = .29; controls: 2.08, *SE* = .29). Analysis of the hemispheres separately revealed a significant effect for the right hemisphere ($F_{1,20}$ = 10.02, p < .005), while there was only a trend for the left hemisphere ($F_{1,20}$ = 2.50, p < .12).

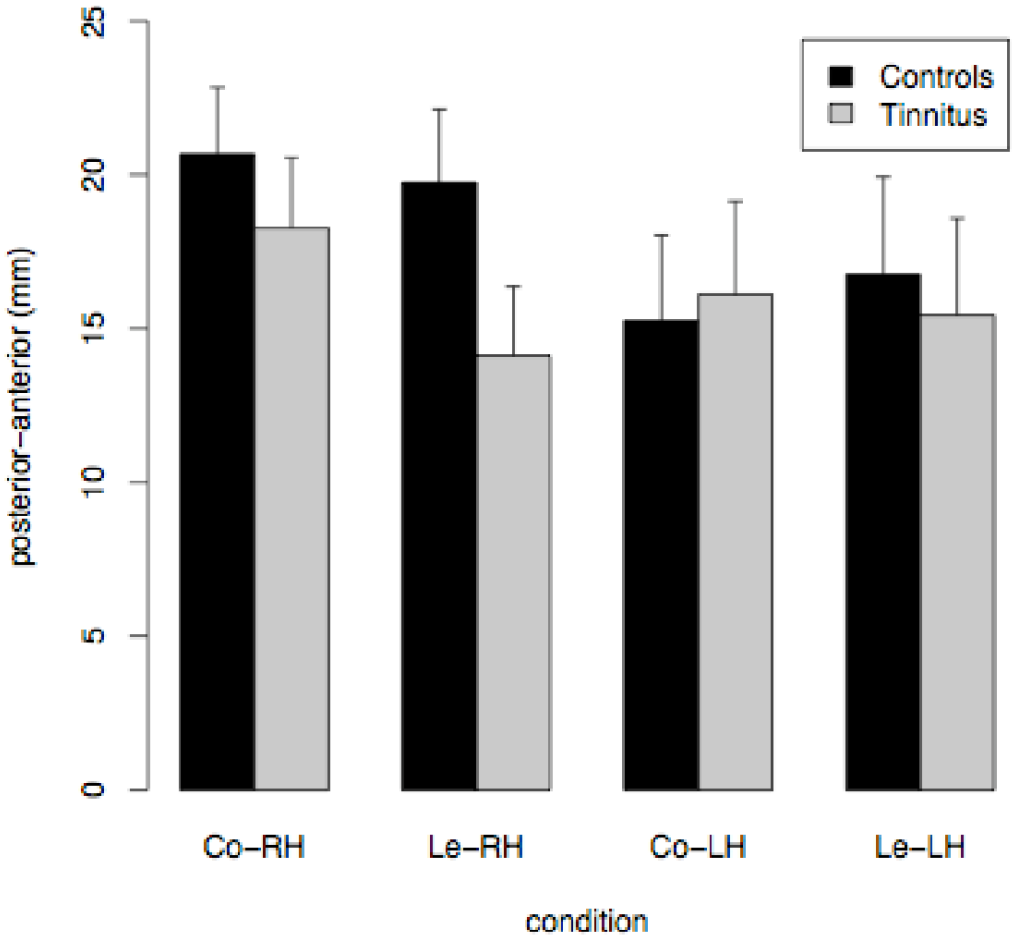

Figure 3-13: Source location on the posterior-anterior axis show almost identical values for the left hemisphere (LH) for the two groups. In comparison to the control group, locations for LE appear to be further posterior in the tinnitus group. As the overall interaction of the repeated measures ANOVA was not significant, this effect was not further elucidated here (also because differences were more pronounced using *D*).

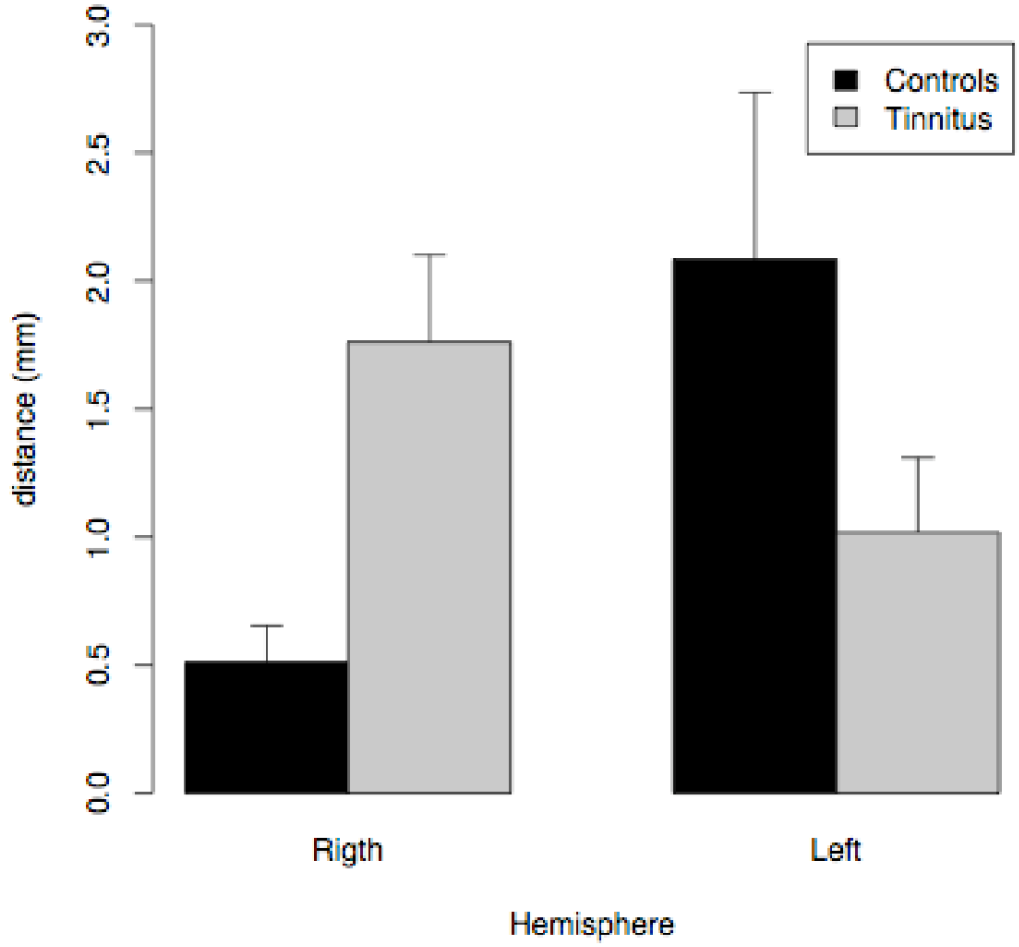

Figure 3-14: *D* values for the posterior-anterior axis (reflecting deviance from identical locations for an axis; the greater the value, the larger the distance between CO and LE on the tonotopic map) show a reversed pattern for the groups. While *D* is greater in the left hemisphere in controls, it is larger in the right hemisphere of tinnitus subjects. The difference between the groups is significant in the right hemisphere, which could indicate expanded tonotopy in the tinnitus group.

Concerning associations with tinnitus related distress *D* showed no significant relationship. Source location in posterior-anterior direction was however very strongly correlated with distress for both conditions in the left hemisphere (CO: $r = .80$, $t_{11} = 4.49$, $p < .001$; LE: $r = .74$, $t_{11} = 3.62$, $p < .004$; see Figure 3-15). Further anterior sources correspond to enhanced distress values. Although statistically not significant, the same tendency existed also for the right hemisphere (both conditions: $r \sim .47$, $t_{12} = 1.8$, $p < .10$). For *D* no substantial correlations could be observed with tinnitus related distress.

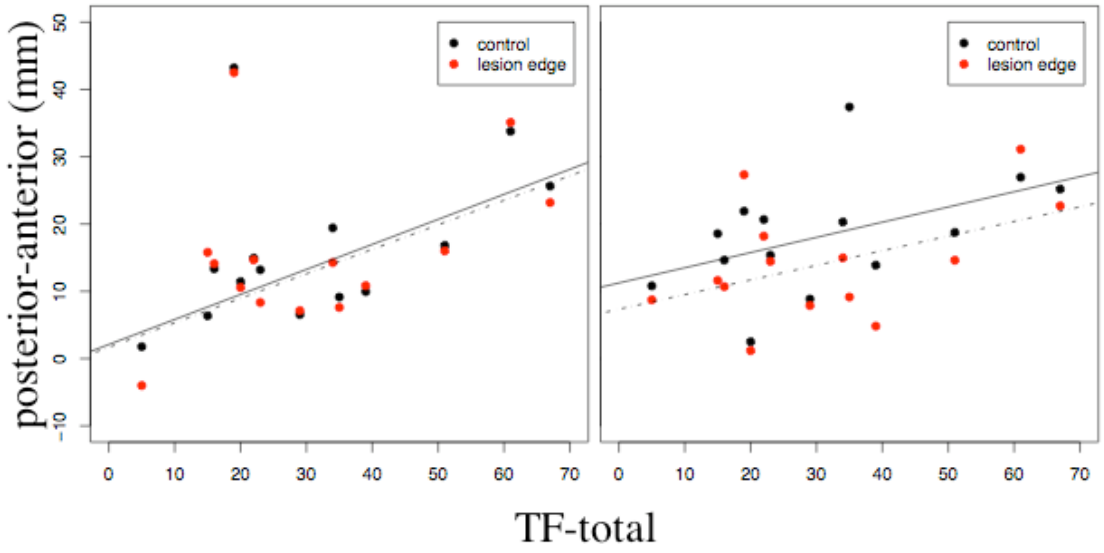

Figure 3-15: A positive association between tinnitus related distress and source location on the posterior-anterior axis was observed for the left hemisphere (left panel; straight line: linear trend for LE; dotted line: linear trend for CO). Although not statistically significant, a similar trend was also observed for the right hemisphere (right panel).

Source location inferior-superior: Average location of sources on the inferior-superior axis ranged between 53 to 55 mm. The repeated measures ANOVA revealed no significant effects. Similarly, no effects were observed with *D* nor were there any relevant associations with tinnitus-related distress.

3.3.4. Discussion

The present study tried to investigate alterations in tonotopic representation and neuronal response dynamics to acoustic stimuli that might accompany tinnitus. A different approach was taken than in the Mühlnickel et al. study (1998) in which hearing loss was regarded as a confounding factor and focus lay on the individual 'tinnitus frequency'. That hearing loss formed an indispensible pillar in regarding tinnitus in this study is reflected in the design in which frequencies were chosen according to the individual audiogram, thus resembling the study done by Dietrich et al. (2001). These authors were able to show great enhancements of the N1m which were specific for the LE frequency, which fit data gained from animal studies (Irvine et al., 2001; Rajan & Irvine, 1998). However, this result could not be replicated here. A possible reason for this discrepancy could lie in the different approach in determining the dipole moment. Dietrich et al. used the value gained from a single moving ECD while the present study applied a fixed source montage. It is known that

source strength can vary considerably with depth of the dipole (which were extremely high on average in the Dietrich et al. study), i.e. it is not known what influence source location might have played in the Dietrich et al. study. Unfortunately the locations were not reported. The present study shows greater responses for CO in the left hemisphere for normal hearing controls. Tinnitus sufferers exhibit this enhancement in the right hemisphere. Another effect involving a hemispheric reversal for the groups concerns the *D* measure (operationalizing distances between LE and CO) for the posterior-anterior direction: while control participants have larger *D* in the left hemisphere, it is in the right hemisphere for tinnitus subjects. These results open up paths for speculations concerning the role of hemispheric asymmetry and also of involvement of interhemispheric plasticity. Here the question remains open whether auditory representational areas in both hemispheres change simultaneously due to a common cause or whether changes in one hemisphere trigger alterations in the other. Speculations about potential differential roles of the hemispheres is furthermore triggered by the negative association between slope of hearing loss in the left ear and tinnitus related distress, which could not be observed for the right ear. A similar effect was already observed in study 2 (Weisz et al., 2004a). It would be interesting to investigate whether the restriction to the left ear has something to do with the fact that left (or left dominant) tinnitus is somewhat more common (Lenarz, 1992). Yet, it would also be necessary to understand what the audiometric hearing loss slope reflects in terms of underlying receptor damage or alteration of the afferent input to auditory brain areas. It is well known that inferences about physiological damage taken from the audiogram are not trivial (Moore, Huss, Vickers, Glasberg, & Alacantra, 2000; Shiomi et al., 1997). Some psychoacoustical studies examining frequency discrimination limens reported finer thresholds at edge frequencies (McDermott et al., 1998; Thai-Van et al., 2003; Thai-Van et al., 2002). The authors usually interpret this result as a consequence of a larger representation of LE, i.e. that more neurons are involved in processing audiometric edge frequencies. This could not be confirmed in our study, at least via MEG. Also, specifically the work by Thai-Van et al. (2002) showed that the effects for frequency discrimination were most prominent in subjects with steep slopes implying that reorganization is most pronounced in these persons. Yet, the slope did not exhibit any associations in this direction. Paradoxically the correlation between tinnitus related distress and slope was

even *negative*. These results cast doubts on the notion that tinnitus may be a result of *simple* reorganization processes in the representation of LE (Rauschecker, 1999). This notion is also strengthened by the condition unspecifity of some effects, such as the generally more lateral location of sources for the tinnitus participants or the strong association between tinnitus related distress and source location on the posterior-anterior axis (the effect being stronger for the left hemisphere). The latter result replicated the positive correlation found in study 2 (Weisz et al., 2004a). However, one LE specific effect was found for the middle-latency component P1m: subjects with high scores on tinnitus related distress expressed earlier peaks for the P1m indicating faster processing of the LE frequency range. Based on the present state of knowledge, it is not clear whether these results reflect the fact that auditory input might be processed by a neural system that is highly synchronized already in a resting state, thus processing the input more efficiently; or whether top-down (attentional and emotional) processes exert a modulatory influence on more basic auditory processing stages. The latter notion could be corroborated study 2, in which a complex mismatch pattern was reported, which was LE specific, or the enhanced negative difference wave (Nd) described by Jacobsen et al. (1996).

The ideas however are not mutually exclusive and might work on different temporal scales: it is well conceivable that auditory cortical neurons show enhanced synchronous activity following deafferentation (Norena & Eggermont, 2003) being responsible for the often perceived sudden onset of the tinnitus perception. This process might be related to a fast unmasking of lateral excitatory input commonly underlying sensory filling in phenomena (Pessoa & De Weerd, 2003). For tinnitus to become chronic it is likely that more permanent neuroplastic processes rather than a reduced lateral inhibition takes place: neuronal networks involved in regulation of attention could activate cholinergic basal forebrain structures (e.g., nucleus basalis) which in turn project to neurons of the auditory cortex triggering plastic processes (Møller, 2003). I will return to this issue in chapter 4.

Although many interesting effects were found, several open questions remain. In my opinion, it is not sensible and hardly feasible to investigate tinnitus *without* a damage to the auditory system of some kind (usually to the receptors of the inner-ear), making it difficult to decipher what mechanisms may be – if at all – causally related to tinnitus and which may be a 'simple' epiphenomenon of hearing loss. This is e.g.

the case for the role of representation of frequencies outside of the hearing loss range (e.g., CO and LE). Do altered responses reflect any involvement in the generation of tinnitus, e.g. by driving synchronous activity in deafferented regions? Or may they be indicative of an altered salience of acoustic input (Willott, 1996; Willott et al., 1994), which could be involved in tinnitus generation more indirectly (via top-down mechanisms)? Approaching a solution will therefore need the combined effort of behavioural and neurophysiological studies in humans and animals.

3.4. Side-Effect: Steepness of hearing loss as audiometric correlate of distress

As described in the introduction there are some authors assuming that certain features of hearing loss determine the extent to which cortical reorganization occurs (Irvine et al., 2001; Rajan, 1998; Thai-Van et al., 2003; Thai-Van et al., 2002).

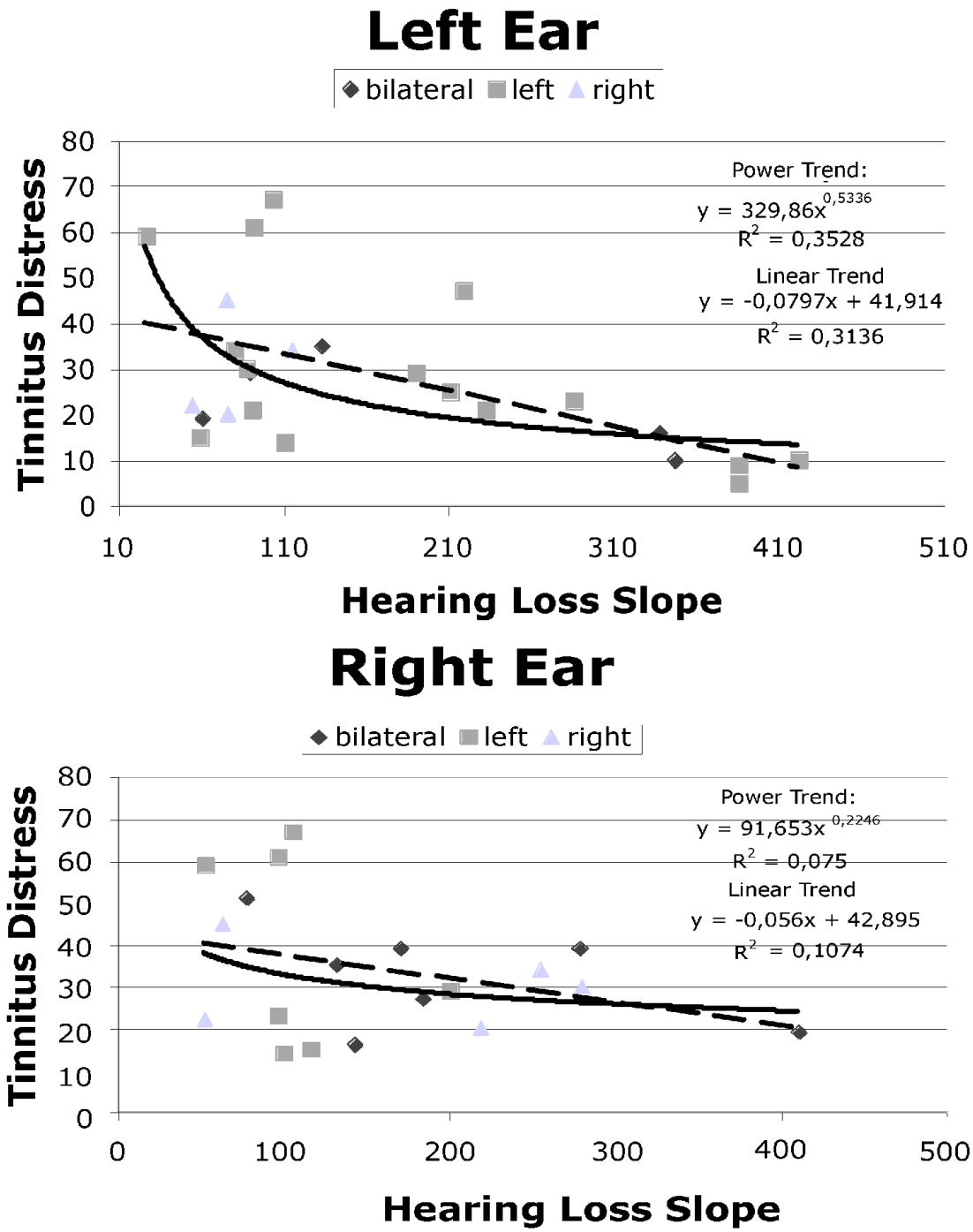

Figure 3-16: Hearing loss slopes data collapsed for different studies plotted against tinnitus related distress. Symbols indicate whether participants perceive their tinnitus unilaterally / dominant on one side (left or right) or bilaterally equal. A strong – positive perhaps nonlinear – association can be seen for the left ear however not for the right ear.

Specifically discussed is an association with depth and steepness of the hearing loss. So these variables were measured too according to the audiogram of the individual and related to the other dependent variables (neurophysiological and distress). An unexpected negative association between slope of hearing loss an tinnitus distress was found across studies specifically for the *left* ear, i.e. the less steep the hearing loss the stronger tinnitus related distress (see Figure 3-16). The only clear correlation between slope and a neurophysiological variable was found with the abnormal mismatch pattern (see study 2). In this case abnormality (associated with lower distress) was associated with steeper slopes. These results are somewhat contrary to the intuitive initial assumption that subjective features of tinnitus are determined by enlargement of lesion-edge frequency representations which are stronger in steeper hearing losses. I will discuss this result in the context of what effect altered auditory filters in the periphery may have on auditory cortical reorganization. No similar effect could be seen for hearing loss depth.

3.5. Summary of main findings

In total, three studies regarding neurophysiological correlates of tinnitus were presented here. The initial hypothesis viewed tinnitus as an auditory analogy of somatosensory phantom pain (map reorganization hypothesis). Study 2 and 3 addressed this issue. The main points were:

- No evidence was found for a general cortical overrepresentation of lesion-edge frequencies. However this does not exclude that some lesion-edge specific processes may play a role: e.g. an abnormal mismatch pattern and faster processing (measured via the P1m) of lesion-edge frequencies could be shown in more distressed subjects.
- Both studies could consistently find a strong positive correlation between distress values and anterior shift of neuronal activity.
- Specifically in the MEG study there were indicators of differences in hemispheric asymmetry between normal hearing controls and tinnitus subjects concerning magnitude and location of neuronal activation. The results were even reversed for some variables. The *D*-values for the posterior-anterior axis suggests enlarged right hemispheric tonotopic representation.

Study 1 was concerned with the question whether neuronal signatures of tinnitus can be found in the ongoing spontaneous activity. The most important results here were:

- Tinnitus participants exhibit bilaterally focal concentrations over temporal regions of delta activity enhancement and alpha activity reduction.
- This pattern of abnormal spontaneous activity correlated strongly with tinnitus related distress for left frontal and right temporal regions.
- Overall, the effect is stronger for alpha than for delta activity.

A side effect of all studies was an unpredicted negative association between hearing loss slope and tinnitus related distress which was pronounced for the left ear. This audiometric variable was positively correlated with abnormality of mismatch pattern.

4. General discussion

In the first part of this chapter I will try to discuss the results in a more integrated manner and based on the background of what has been described in the introduction. The main aspects were: 1) tinnitus is a consequence following map reorganization similar to somatosensory phantom-limb pain (section 4.1.). And 2) tinnitus is a consequence of altered spontaneous activity (section 4.2.). Later (section 4.3.) I will discuss a hypothesis how changes in *peripheral* excitation pattern could drive to some extent central reorganization (map reorganization and spontaneous activity). At the end of this chapter I will try to integrate these works and those of others into a working model of tinnitus (section 4.4.).

4.1. Tinnitus a consequence of map reorganization?

If tinnitus really was an auditory analogue of phantom limb pain (Elbert et al., 1994; Flor et al., 1995) as suggested by some authors (Irvine et al., 2001; Mühlnickel et al., 1998; Rauschecker, 1999) then stimulation with lesion-edge frequencies should go along with increased neuronal responses or spatial shifts of neuronal activity. This was neither the case for the MEG (study 3) nor the EEG study (study 2), and stands in contradiction to the results reported by Dietrich et al. (2001). It is difficult to figure out why this discrepancy exists, as the designs were very similar. A possible explanation mentioned earlier could lie in different approaches how source activity was modeled. The negative finding in this point however does not exclude that abnormal lesion-edge processes occur. On the contrary these studies have shown neurophysiological responses specific to lesion-edge stimulation that were associated with tinnitus related distress, among other things a faster processing (study 3) and an abnormal mismatch pattern (study 2). While faster P1m latencies could be indicative of primary auditory cortical map plasticity (e.g., enhanced synchronous activity in a resting state), the abnormal mismatch pattern is hardly compatible with such a notion. First of all there is the complexity of the neurophysiological response pattern including enhancement *and* reduction. Argumentation with potential peripheral (cochlear) mechanisms seems pointless, as the individual lesion-edge is currently determined by visual inspection from the audiogram which does not offer precise information about the properties of the damage. This also precludes possible points

that could be raised by adherers of a 'tinnitus frequency' view (Diesch et al., 2004; Kadner et al., 2002; Mühlnickel et al., 1998), that an overexcited lesion-edge region (in which a 1% deviant would fall) inhibits neighbouring regions (in which a 2% deviant would fall). This argument is implausible due to the small frequency differences (20 Hz in case of a 2000 Hz lesion-edge frequency), and even if correct the method used to determine the lesion-edge frequency does not offer such a resolution. Also, correlations showed that steep hearing loss slopes were associated to *less* distress, contrary to what should be expected if tinnitus was a consequence of map reorganization (assumed to be positively correlated with slope; McDermott et al., 1998; Rajan, 1998; Thai-Van et al., 2003; Thai-Van et al., 2002). Furthermore, with increasing distress neuronal activity is shifted towards anterior locations in both studies. The scatter of locations of approximately 3-5 cm make an exclusive involvement of auditory cortical areas improbable. So even though there is data for map reorganization in tinnitus, e.g. a more lateral source location or enhanced right hemispheric distance between lesion-edge and control frequency representation (*D*; study 3), I do not regard this as convincing evidence for a causal involvement of map reorganization in tinnitus. Thus I currently view the neurophysiological associations with distress to reflect top-down modulation of auditory cortical activity from brain regions responsible for emotional and attentional regulation. Clues for this assumption are given by indicators of frontal cortex involvement (right in study 2; left in study 3, see next section) and that the time-window for effects in the Mismatch study is almost identical to the one reported by Jacobsen et al. (1996) for the Nd-wave.

To conclude: **even though some lesion-edge specific effects were found across studies, they were not so straight forward as when tinnitus was a one to one auditory analogue of phantom limb pain**. The general pattern is too complex to only indicate mere map reorganization of lesion-edge neurons.

4.2. Tinnitus a consequence of altered spontaneous activity?

Naturally, only a few aspects of spontaneous activity can be investigated with MEG which can be accomplished with invasive single or multiple unit recordings (e.g., Norena & Eggermont, 2003). However if abnormal thalamocortical bursting activity underlies tinnitus (Jeanmonod et al., 1996), then it should be possible to pick up oscillatory activity in a frequency range corresponding to the bursting rythm with

neuromagnetic recordings. Indeed analysis of the MEG signal revealed bilateral focal enhancements of slow waves (~2.5-4 Hz) and an accompanying reduction of alpha activity in tinnitus subjects in temporal regions. The negative relationship between slow wave activity and alpha activity has already been reported earlier (Benoit et al., 2000; for theta see Llinas et al., 1999). The left frontal / right temporal association of this deviant spontaneous activity pattern with tinnitus distress values shows, that it could be of relevance for tinnitus. The possibility that the frontal alpha asymmetry simply reflects enhanced depression in the tinnitus group can be excluded due to the direction of the association: work by others (Davidson & Irwin, 1999; Davidson et al., 2002; Wheeler et al., 1993) and in Konstanz (Wienbruch et al., 2003; Slapin, unpublished data) show a positive correlation, whereas the one reported here is negative. The fact that the association with distress was stronger for right rather than left temporal areas could reflect the simple fact that the great majority of our subjects report left or left dominant tinnitus when lateralized. It could however also reflect principal hemispheric differences regarding the importance for tinnitus. I will discuss this issue at a later point (section 4.4.). Although causal evidence is lacking, it seems plausible to assume that changes in **spontaneous activity in temporal areas reflect perceptual aspects**, perhaps also mediated via enhanced gamma activity (not investigated in our study) that arises in regions between abnormal and normal awake activity ('edge-effect'; Llinas et al., 1998). **The left frontal region may be related more with the affective distress and motivational attention of tinnitus. Slow oscillatory activity, known to be typicial for long range cortical coherence (Llinas et al., 1998), could be the underlying neurophysiological mode by which temporal and frontal areas are coupled together into a tinnitus-related network**.

4.3. Periphery and Plasticity

If an association between hearing loss slope and auditory cortex reorganization (and therefore tinnitus) would have been proposed prior to the studies, then the map reorganization approach to tinnitus would predict a positive correlation (McDermott et al., 1998; Thai-Van et al., 2003; Thai-Van et al., 2002): i.e. the steeper the slope the stronger neuroplastic effects and subjective indicators of tinnitus. Partly because of the completely opposite results, the opportunity was taken to reconsider the initial notion that injury-induced cortical reorganization is a autonomous process on a

central level once a damage has been applied to peripheral receptors. The main concepts in this framework – loss of surround inhibition and change of synaptic strength between neurons due to an altered behavioural relevance – have been described in section 1.2.. However, it does not seem sufficient at least for the auditory modality to propose *solely* such mechanisms. ***More likely is that continuous input from a damaged auditory system will continue to shape functioning of neurons in relevant representational areas***: i.e., a kind of maladaptive use-induced plasticity following injury-induced reorganization of auditory cortical areas.

Since Fletcher's (1940; cf. Moore, 2003) pioneering work on critical bands, the basilar membrane is viewed as a bank of overlapping bandpass filters (so-called auditory filters): frequency selectivity is thus already reached on the most peripheral level. In case of 'perfect' frequency selectivity only a single auditory filter would be involved in detecting a pure tone. However, this is not the case as 1) multiple filters are activated by a pure tone and b) detection tasks may be better solved by attending to neighbouring filters. The latter phenomenon is also known as *off-frequency listening* and can be determined e.g. by psychoacoustical tuning curves (PTC). The neural 'excitation pattern' is determined by the output of several auditory filters in response to a tone and is critically dependent on the shape of the involved filters (Moore & Glasberg, 1983). As bandwidths of filters increase proportionally with increasing center frequency (CF), more filters with higher CFs are activated, leading to a broader excitation on the high frequency side (see Figure 4-1b). In cases of cochlear damage, **frequency selectivity is known to be significantly altered in the affected regions** (Moore, 1995). One aspect is that **auditory filter broaden in this range leading to an enhanced neural excitation pattern**. This is largely dependent on the damage to outer hair cells. The second aspect concerns especially the presence of so-called dead regions, i.e. regions on the cochlea with no functioning inner hair cells (Moore et al., 2000): detection of frequencies is accomplished with neighbouring undamaged hair cells (the just mentioned **off-frequency listening**). Filter broadening and off-frequency listening can be seen by investigating PTCs (see Figure 4-1a) . This is relevant for reorganization, because it means that ***auditory input to lesioned and perilesioned areas will lead to different neuronal processing determined already on a very peripheral level***. This is exemplified for a stimulation at the audiometric lesion-edge in Figure 4-1b , in which the excitation pattern in hearing impaired

persons is enhanced. It can be expected that *continuing input can contribute via a broader excitation pattern to modifications of receptive fields* leading to the wide-spread map reorganization described above. Frequent *co-activation of representational neurons due to this altered excitation pattern could also account for some of the changes seen in spontaneous activity*, specifically coherence of deafferented neurons. To our knowledge the only work addressing the question of auditory filters in tinnitus directly are those conducted by Cazals (2000) and Dauman and Cazals (1989). These works demonstrate a broadening of filters for frequencies matched to the tinnitus as compared to hearing impaired ears without tinnitus. Unfortunately, auditory neuroscience has so far not reflected enough about what this could mean in terms of cortical reorganization following injury to the cochlea. Shallow slopes could partly reflect cochlear regions with extreme broadening of auditory filters. Stimulation would thus lead to an output from more filters than normal, which in turn synchronously activates deafferented regions of the auditory cortex.

Another important implication concerns therapeutic approaches that attempt to drive back assumed maladaptive reorganization (see section 1.2.3.) by means of a behavioural training, usually operationalized as a frequency discrimination training (including the own first attempts made in Konstanz): These approaches postulate a critical frequency region (e.g., close to the 'tinnitus frequency' or inside the hearing loss) in which behaviourally relevant stimulation has to be processed. They implicitly assume that it can be done with great accuracy. This assumption however is **wrong**, because it ignores the fact that the **auditory information that reaches the brain of a hearing impaired subject will be different – due to the peripheral mechanisms described above – from that of a normal hearing person**. The ideal approach would be to assess psychological tuning curves (PTCs) over a very wide range of frequencies, in order to estimate excitation characteristics and the amount of off-frequency listening: in practice however this would be an impossible undertaking due to the long duration it takes to measure PTCs (most psychoacoustic experiments do not measure more than about 3). Some fast tools exist for specific aspects (for example the threshold equalizing noise, TEN, approach for off-frequency listening; Moore et al., 2000), but still there is no all-embracing diagnostic approach for this problem. Before they are developed however, **behavioural training approaches**

should nevertheless proceed with a more sophisticated use of noise in order to suppress as much as possible the undesired inclusion of neighbouring filters in solving the task (e.g., a discrimination or a threshold).

4.4. A temporary working model

In this thesis I presented abundant basic evidence for an altered neurophysiological functioning in circumscribed areas involving temporal (auditory) and frontal cortex. Yet it is unclear to pinpoint which changes (if any) may reflect a causal involvement in the generation, maintainance and related emotional distress or whether all measures may be an epiphenomenon of a common underlying mechanism. So far there is no absolutely convincing empirical evidence that tinnitus could be an auditory analogue of somatosensory phantom pain, which was the initial hypothesis at the beginning of the project. At the moment it is likely that different mechanisms are active on different temporal scales (this notion partly rests upon the data presented in this thesis or studies by others described in the introduction, and partly – due to the small body of secured knowledge on tinnitus – speculation; ideas summarized in Figure 4-2 which follows strongly descriptions given by Møller (2003) and Weinberger (1998)). Due to simplicity the following description will focus on the 'last link' of mechanisms that could be associated with the perception of tinnitus and its distress. It explicitely does not exclude the possibility of other subcortical influences (inferior colliculus, dorsal cochlear nucleus; Kaltenbach et al., 2004; Wallhäusser-Franke et al., 1996; Wallhäusser-Franke et al., 2003).

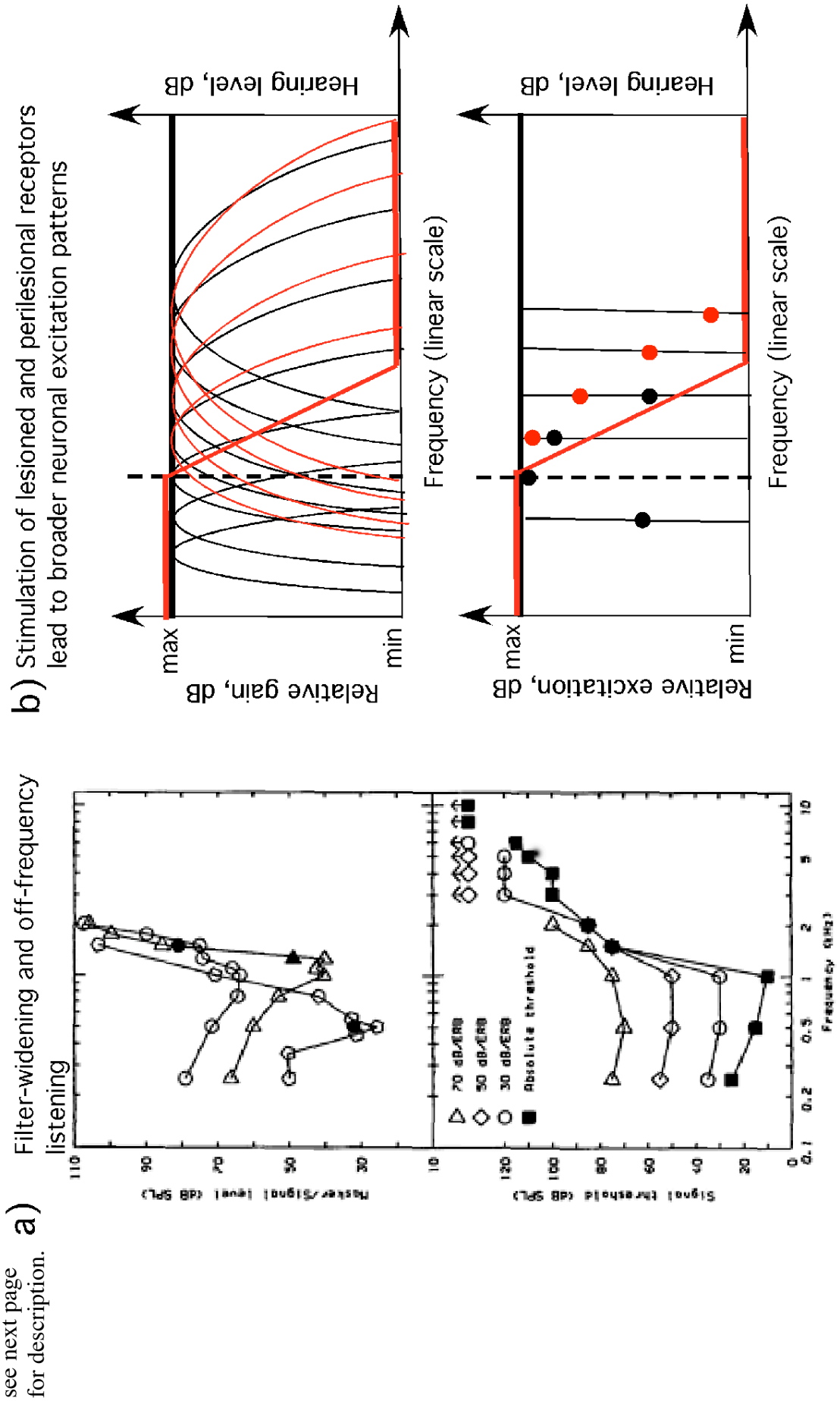

Figure 4-1: see next page for description.

Figure 4-1 (cont.): a) Cochlear damage is associated with a widening of auditory filters in damaged regions, which can be seen in the uppermost PTC in the upper panel. Furthermore the tip of this tuning curve is significantly shifted away from the stimulation frequency (~ 1.5 kHz) to lesion-edge frequencies (~ 1 kHz), an example for 'off-frequency' listening. b) This is a schematic example (adapted from Moore, 2003) how stimulation of audiometric edge frequencies is associated with broadened excitation patterns. The thick black line represents normal hearing level and the black parabola proposed auditory filters for various CFs. The black points in the lower panel show the output a filter, when a stimulation at a certain frequency is given (hatched line). Due to filter widening in the impaired regions (red parabola, upper panel) more auditory filters yield output leading to a broader excitation pattern in the deafferented regions. This mechanism could be involved in map reorganization and an enhanced coherent firing of deafferented neurons.

- *Immediately* (within seconds) following a damage to the hearing system – e.g., noise trauma, sudden hearing loss etc. – a loss of surround inhibition within deafferented areas of the auditory cortex leads to an **enhanced coherent firing of neurons**. This is perceptually reflected in the rapid onset of the tinnitus sensation (Norena & Eggermont, 2003). Later slow thalamocortical bursts with potentially enhanced 'edge-effect' gamma activation could stabilize this process (Jeanmonod et al., 1996; Llinas et al., 1998; Llinas et al., 1999). However, the tinnitus related network definitely goes beyond thalamocortical interactions, since the tinnitus symptomatic appears to be relatively therapy-resistent against medial thalatomy (Jeanmonod et al., 1996; see section 1.2.3.) At this point I would like to emphasize again, that **this approach to tinnitus sees a physiological (perhaps temporary) impairment as absolutely necessary for the development of the neuronal processes that lead to tinnitus perception**. This judgement is based on epidemiological data (Pilgramm et al., 1999; Weinberger, 1998) and the fact that also in the great majority of seemingly audiometrically unimpaired patients a damage to outer hair cell functioning can be verified (Shiomi et al., 1997).
- On a *short- and medium-term*, due to the negative impact on daily functioning or the overall negative context in which the tinnitus emerged (several patients report stressing situations) the tinnitus perception obtains the status of a sensation of enhanced **behavioural relevance**. These processes presumably mediated by limbic structures (Lockwood, Salvi, Coad, Arnold, Wack, Murphy, & Burkhard, 1999; Lockwood et al., 1998; Mirz et al., 2000; Mirz et al., 1999) could boost maladaptive auditory cortical reorganization and could

be responsible for the highly interindividual affective side of tinnitus. The connection between limbic areas and cholinergic areas with its important involvement in cortical reorganization has been elaborated on in section 1.2.4. (Weinberger, 1998). Brain areas responsible for the **basic auditory and attentional-emotional processing could be coupled** via slow oscillatory brain rythms. This could be partly mediated by processes in the secondary auditory cortex, shown to have increased spontaneous firing rate following hearing damage (Eggermont & Kenmochi, 1998). In this context, it is also worth mentioning again that some **hemispheric asymmetry could play – at least a modulatory – role in tinnitus**. Next to our data (e.g. 'reversed' results concerning dipole strength and localization in posterior-anterior direction; enhanced D potentially reflecting enhanced tonotopic representation; alpha reduction / delta enhancement) this is also hinted by epidemiological data, e.g. more frequent left than right sided / dominant tinnitus (Axelsson & Ringdahl, 1989; Stouffer & Tyler, 1990), tinnitus perceived as more disturbing in the left ear (see www.tinnitusarchive.org; Meikle, 1997), men (except at higher ages, > 69 years) more affected than women (Axelsson & Ringdahl, 1989; Stouffer & Tyler, 1990). The gender difference is frequently explained by a different noise exposure of men and women, however with the lack of offering empirical support for this notion. Also Axelsson and Ringdahl report a more consistent association with hearing loss reported on the left side and left-laterality of tinnitus perception than for the right ear. The latter aspect is corroborated by our finding of a stronger association between hearing loss slope and tinnitus distress for the left ear. At this stage it is completely unclear – yet intuitively appealing – whether this has to do with the frequently proposed stronger involvement of the right hemisphere in processing emotional content of stimuli (e.g., Atchley, Ilardi, & Enloe, 2003; Kayser, Tenke, Nordby, Hammerborg, Hugdahl, & Erdmann, 1997; Nakamura et al., 1999). In a resting state stronger associations with distress can be seen for the left hemisphere: the functional meaning of an alpha reduction / delta enhancement can not be derived from the present data, however they do not fit the frontal alpha asymmetry hypothesis for depression put forward by Davidson and colleagues (Davidson & Irwin, 1999; Davidson et al., 2002).

- Also on a *medium- to long-term* altered input due to **filter-widening and effective broadened excitation leads to an enhancement of synchronous activation of deafferented neurons** (perhaps together with lesion-edge representational neurons), resembling a negative form of use-induced plasticity. **Off-frequency listening in cases of inner hair-cell damage leads to an increased processing of lesion-edge frequencies** (see Figure 4-1a).

Certainly these aspects described here can only offer a starting point for further research, and should not be misunderstood as a definite model. Future studies in this field will have to (re-)confirm several of the mechanisms proposed here and address the question **why** they exist (i.e., development and function).

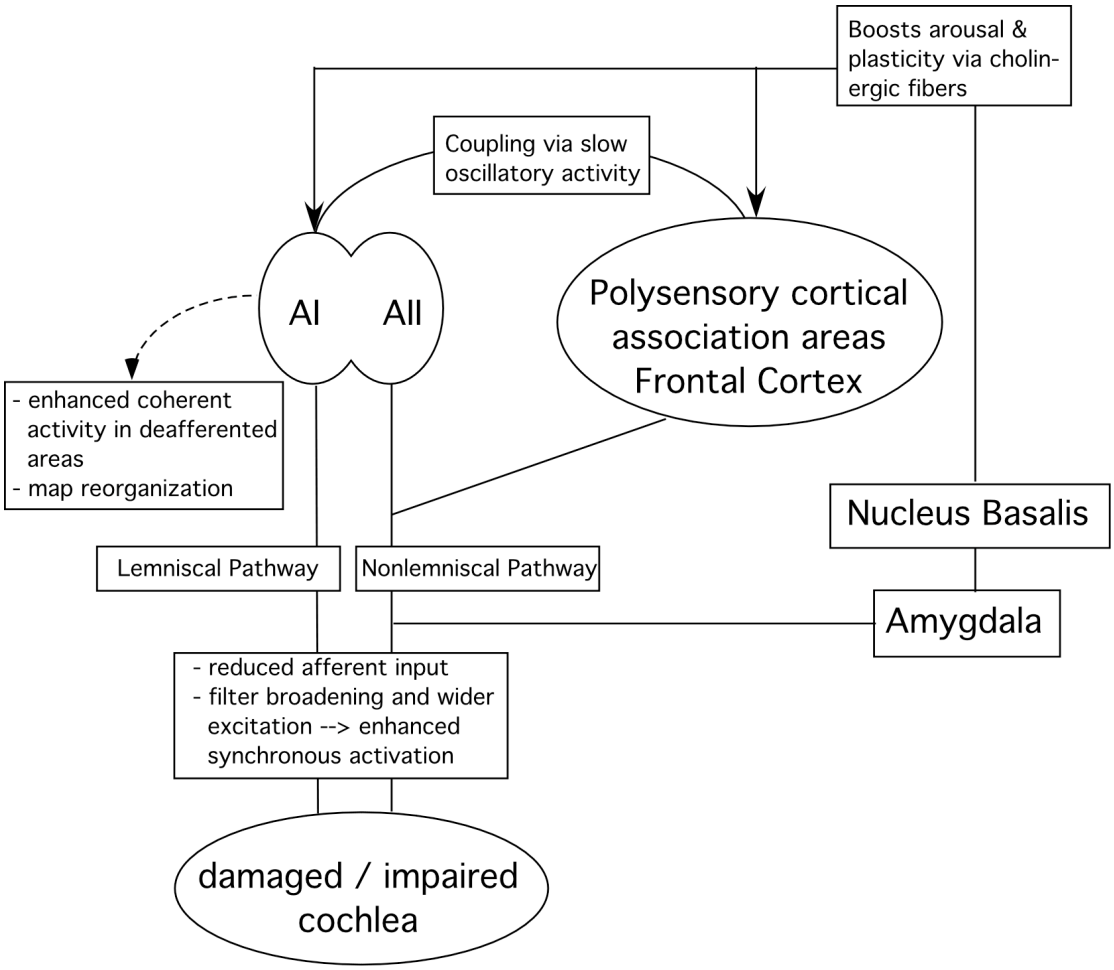

Figure 4-2: The graphic follows largely a diagram given by Møller (2003, p. 255), without however specifying most of the subcortical (e.g., thalamic) details. Also two aspects have been added (altered excitation pattern due to filter-widening; slow oscillatory activity) due to results from the studies presented previously. The figure basically summarizes some of the important ideas about tinnitus, in which we have

tried to integrate our findings. The basis for the development of tinnitus is a peripheral damage (usually cochlear origin). Reduced afferent input to certain regions of the primary auditory cortex (AI) leads to an increase of correlated activity in those areas. This process is enforced by a distorted peripheral sound transmission. Input from nonauditory limbic areas boost these neuroplastic processes and are responsible for the subjective distress. Cortical (especially frontal) areas involved in emotional and attentional regulation are coupled with auditory regions via slow oscillatory activity.

5. Perspectives

Tinnitus will continue to be a plague for a large number of people unless more insight is gained into the neurophysiological mechanisms underlying this puzzling auditory phenomenon. Certainly at this stage where so little is understood about tinnitus, some of the ideas described in this thesis may turn out to be wrong, but several results also open up interesting perspectives for research in the near future. I will end this dissertation by naming – from my subjective viewpoint – the 5 most important research questions (order does not imply a ranking of importance) emerging from the work presented here:

1. A large amount of the data indicates that auditory information in subjects with hearing loss and tinnitus is neuronally not processed in the same manner as in normal hearing control subjects. It could also be seen that many effects can not be explained by 'simple' distortions of tonotopic representation but that presumably top-down (frontally) mediated processes play a role. **This raises the question whether auditory information (simple and complex) has an altered behavioural relevance and whether this would be reflected by behavior**. So far the studies have been very basic, incorporating simple sine tones (or even no stimulation) and requiring no response. Currently attempts are being undertaken to compare tinnitus and normal hearing controls on various cognitive tasks requiring processing of acoustic information with *undamaged* cochlear regions.
2. Some of our data reflect distinct hemispheric differences in auditory cortical representation between tinnitus an normal hearing controls. Also, the general notion is that tinnitus is localized contralateral to the hearing loss side. However, far more left or left dominant tinnitus than right lateralized tinnitus was observed even in cases with bilateral hearing loss. It is noteworthy to mention again that associations with hearing loss slope are stronger for the left ear. Furthermore, more male than female subjects (independent of age) enrol in our studies, even in current ones with students which is highly unusual in psychology. **All these details give opportunity to speculate that besides of peripheral damage, psychoacoustic (e.g. laterality) and perhaps psychological features (e.g. distress) could also be determined by**

hemispheric specializations. The role of hemispheric asymmetry has only played a minor role so far.

3. A very important question which needs to be addressed is **which influence, besides of 'purely' central processes, auditory information mediated by a damaged peripheral system has on shaping response properties of auditory cortical neurons**. Even though filter broadening and expanded excitation have been known in psychoacoustics for a long time this has not been reflected enough in auditory neuroscience. Because auditory input seldomly ceases completely, a distorted input from the auditory periphery should alter auditory cortical functioning in a use-induced manner. If tinnitus really was somehow causally related to reorganization of the auditory cortex, then the signal transmission properties of an impaired periphery would have to be considered in treatment attempts either via hearing trainings or the fitting of hearing aids.
4. Although psychoacoustic features of tinnitus vary surprisingly little between affected persons (usually described as high tonal sensation, matched close to hearing threshold), the distress related to tinnitus shows a very strong variation. More effort will be needed to find out **which psychological and neurophysiological processes mediate tinnitus related distress**.
5. It has been mentioned already in the introduction that the success of neuroscientfic approaches to tinnitus will be judged someday by their ability to ***yield therapeutically useful information*** **on a medium-term**. A successful therapy would need to be based on a theory that finds answers to several of the questions mentioned in this thesis: e.g., the role & nature of tinnitus related reorganization in general, the influence of altered peripheral input on reorganization, hemispheric specialization, top-down influences and distress-mediating variables. Neuroscientists have to understand that the high claim to search for *causal* mechanisms raises hopes for a *causal* therapy. Currently we have a long way ahead of us.

References

Ahissar, E., Abeles, M., Ahissar, M., Haidarliu, S., & Vaadia, E. (1998). Hebbian-like functional plasticity in the auditory cortex of the behaving monkey. *Neuropharmacology*, *37*, 633-55.

Ahissar, E., & Ahissar, M. (1994). Plasticity in auditory cortical circuitry. *Curr Opin Neurobiol*, *4*, 580-7.

Ahissar, E., Vaadia, E., Ahissar, M., Bergman, H., Arieli, A., & Abeles, M. (1992). Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. *Science*, *257*, 1412-5.

Alho, K., Woods, D.L., Algazi, A., Knight, R.T., & Naatanen, R. (1994). Lesions of frontal cortex diminish the auditory mismatch negativity. *Electroencephalogr Clin Neurophysiol*, *91*, 353-62.

Atchley, R.A., Ilardi, S.S., & Enloe, A. (2003). Hemispheric asymmetry in the processing of emotional content in word meanings: the effect of current and past depression. *Brain Lang*, *84*, 105-19.

Axelsson, A., & Ringdahl, A. (1989). Tinnitus--a study of its prevalence and characteristics. *Br J Audiol*, *23*, 53-62.

Bao, S., Chan, V.T., & Merzenich, M.M. (2001). Cortical remodelling induced by activity of ventral tegmental dopamine neurons. *Nature*, *412*, 79-83.

Benoit, O., Daurat, A., & Prado, J. (2000). Slow (0.7-2 Hz) and fast (2-4 Hz) delta components are differently correlated to theta, alpha and beta frequency bands during NREM sleep. *Clin Neurophysiol*, *111*, 2103-6.

Berg, P., & Scherg, M. (1994). A multiple source approach to the correction of eye artifacts. *Electroencephalogr Clin Neurophysiol*, *90*, 229-41.

Brozoski, T.J., Bauer, C.A., & Caspary, D.M. (2002). Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J Neurosci*, *22*, 2383-90.

Buonomano, D.V., & Merzenich, M.M. (1998). Cortical plasticity: from synapses to maps. *Annu Rev Neurosci*, *21*, 149-86.

Burns, E.M. (1984). A comparison of variability among measurements of subjective tinnitus and objective stimuli. *Audiology*, *23*(4), 426-40.

Cahani, M., Paul, G., & Shahar, A. (1984). Tinnitus asymmetry. *Audiology*, *23*, 127-35.

Calford, M.B., Rajan, R., & Irvine, D.R. (1993). Rapid changes in the frequency tuning of neurons in cat auditory cortex resulting from pure-tone-induced temporary threshold shift. *Neuroscience*, *55*, 953-64.

Canive, J.M., Lewine, J.D., Edgar, J.C., Davis, J.T., Miller, G.A., Torres, F., & Tuason, V.B. (1998). Spontaneous brain magnetic activity in schizophrenia patients treated with aripiprazole. *Psychopharmacol Bull*, *34*, 101-5.

Canive, J.M., Lewine, J.D., Edgar, J.C., Davis, J.T., Torres, F., Roberts, B., Graeber, D., Orrison, W.W., Jr., & Tuason, V.B. (1996). Magnetoencephalographic assessment of spontaneous brain activity in schizophrenia. *Psychopharmacol Bull*, *32*, 741-50.

Cazals, Y. (2000). Auditory sensori-neural alterations induced by salicylate. *Prog Neurobiol*, *62*, 583-631.

Cazals, Y., & Dauman, R. (1990). Tinnitus deteriorates frequency selectivity in addition to hearing loss. In C.G. Grandori F, Kemp DT (Ed.), *Cochlear Mechanisms and Otoacoustic emissions (vol. 7)* (pp. 201-205).

Chermak, G.D., & Dengerink, J.E. (1987). Characteristics of temporary noise-induced tinnitus in male and female subjects. *Scand Audiol*, *16*, 67-73.

Dauman, R., & Cazals, Y. (1989). Auditory frequency selectivity and tinnitus. *Arch Otorhinolaryngol*, *246*, 253-255.

Davidson, R.J., & Irwin, W. (1999). The functional neuroanatomy of emotion and affective style. *Trends Cogn Sci*, *3*, 11-21.

Davidson, R.J., Pizzagalli, D., Nitschke, J.B., & Putnam, K. (2002). Depression: perspectives from affective neuroscience. *Annu Rev Psychol*, *53*, 545-74.

de Jongh, A., de Munck, J.C., Baayen, J.C., Jonkman, E.J., Heethaar, R.M., & van Dijk, B.W. (2001). The localization of spontaneous brain activity: first results in patients with cerebral tumors. *Clin Neurophysiol*, *112*, 378-85.

Diesch, D., Struve, M., Rupp, A., Ritter, S., Hülse, M., & Flor, H. (2004). Enhancement of steady-state auditory evoked magnetic fields in tinnitus. *Eur J Neurosci*, *19*, 1093-1104.

Dietrich, V., Nieschalk, M., Stoll, W., Rajan, R., & Pantev, C. (2001). Cortical reorganization in patients with high frequency cochlear hearing loss. *Hear Res*, *158*, 95-101.

Douek, E. (1987). *Tinnitus following surgery*. Paper presented at the Third Int. Tinnitus Seminar, Münster.

Edeline, J.M., Pham, P., & Weinberger, N.M. (1993). Rapid development of learning-induced receptive field plasticity in the auditory cortex. *Behav Neurosci*, *107*, 539-51.

Edeline, J.M., & Weinberger, N.M. (1993). Receptive field plasticity in the auditory cortex during frequency discrimination training: selective retuning independent of task difficulty. *Behav Neurosci*, *107*, 82-103.

Eggermont, J.J. (1990). On the pathophysiology of tinnitus; a review and a peripheral model. *Hear Res*, *48*, 111-23.

Eggermont, J.J., & Kenmochi, M. (1998). Salicylate and quinine selectively increase spontaneous firing rates in secondary auditory cortex. *Hear Res*, *117*, 149-60.

Eggermont, J.J., & Komiya, H. (2000). Moderate noise trauma in juvenile cats results in profound cortical topographic map changes in adulthood. *Hear Res*, *142*, 89-101.

Eichhammer, P., Langguth, B., Marienhagen, J., Kleinjung, T., & Hajak, G. (2003). Neuronavigated repetitive transcranial magnetic stimulation in patients with tinnitus: a short case series. *Biol Psychiatry*, *54*, 862-5.

Elbert, T. (1998). Neuromagnetism. In W. Andrä & H. Nowak (Eds.), *Magnetism in medicine: a handbook* (pp. 190-261). Berlin: Wiley-VCH.

Elbert, T., Candia, V., Altenmuller, E., Rau, H., Sterr, A., Rockstroh, B., Pantev, C., & Taub, E. (1998). Alteration of digital representations in somatosensory cortex in focal hand dystonia. *Neuroreport*, *9*, 3571-5.

Elbert, T., Flor, H., Birbaumer, N., Knecht, S., Hampson, S., Larbig, W., & Taub, E. (1994). Extensive reorganization of the somatosensory cortex in adult humans after nervous system injury. *Neuroreport*, *5*, 2593-7.

Elbert, T., & Heim, S. (2001). A light and a dark side. *Nature*, *411*(6834), 139.

Evans, E.F., Wilson, J.P., & Borerwe, T.A. (1981). Animal models of tinnitus. *Ciba Found Symp*, *85*, 108-38.

Feldmann, H. (1992). Medizinhistorisches und Kulturhistorisches zum Tinnitus. In H. Feldmann (Ed.), *Tinnitus* (pp. 1-32). Stuttgart: Georg Thieme.

Flor, H., Hoffmann, D., Struve, M., & Diesch, E. (2004). Auditory siscrimination training for the treatment of tinnitus. *Applied Psychophysiology and Biofeedback*, 29, in press.

Flor, H. (2002). Phantom-limb pain: characteristics, causes, and treatment. *Lancet Neurol*, *1*, 182-9.

Flor, H., Elbert, T., Knecht, S., Wienbruch, C., Pantev, C., Birbaumer, N., Larbig, W., & Taub, E. (1995). Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature*, *375*, 482-4.

Frégnac, Y. (1996). Dynamics of functional connectivity in visual cortical networks: an overview. *J Physiol Paris*, *90*, 113-39.

Fritz, J., Shamma, S., Elhilali, M., & Klein, D. (2003). Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex. *Nat Neurosci*, *6*, 1216-23.

Gao, E., & Suga, N. (1998). Experience-dependent corticofugal adjustment of midbrain frequency map in bat auditory system. *Proc Natl Acad Sci U S A*, *95*, 12663-70.

Gao, E., & Suga, N. (2000). Experience-dependent plasticity in the auditory cortex and the inferior colliculus of bats: role of the corticofugal system. *Proc Natl Acad Sci U S A*, *97*, 8081-6.

Gath, I., & Bar-On, E. (1983). Classical sleep stages and the spectral content of the EEG signal. *Int J Neurosci*, *22*, 147-55.

Goebel, G., & Hiller, W. (1998). *Tinnitus-Fragebogen (TF): Ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus*. Göttingen: Hogrefe.

Goldreich, D., & Kanics, I.M. (2003). Tactile acuity is enhanced in blindness. *J Neurosci*, *23*, 3439-45.

Hallam, R.S., Jakes, S.C., & Hinchcliffe, R. (1988). Cognitive variables in tinnitus annoyance. *Br J Clin Psychol*, *27 (Pt 3)*, 213-22.

Hämäläinen, M., Hari, R., Ilmoniemi, R.J., Knuutila, J., & Lounasmaa, O.V. (1993). Magnetencephalography - theory, instrumentation, and applications to noninvasive studies of the working human brain. *Reviews of Modern Physics*, *65*, 413-497.

Hämäläinen, M., & Ilmoniemi, R.J. (1994). *Interpreting measured magnetic fields of the brain: minimum norm estimates*. Helsinki: Helsinki University of Technology.

Hauk, O., Keil, A., Elbert, T., & Müller, M.M. (2002). Comparison of data transformation procedures to enhance topographical accuracy in time-series analysis of the human EEG. *J Neurosci Methods*, *113*, 111-22.

Hebb, D.O. (1949). *The organization of behavior: a neuropsychological theory*. New York: Wiley.

Heffner, H.E., & Harrington, I.A. (2002). Tinnitus in hamsters following exposure to intense sound. *Hear Res*, *170*, 83-95.

Henry, J.A., & Meikle, M.B. (2000). Psychoacoustic measures of tinnitus. *J Am Acad Audiol*, *11*, 138-55.

House, J.W., & Brackmann, D.E. (1981). Tinnitus: surgical treatment. *Ciba Found Symp*, *85*, 204-16.

Huber, P. (1964). Robust Estimation of a Location Parameter. *Annals of Mathematical Statistics*, *35*, 73-101.

Irvine, D.R., Rajan, R., & Brown, M. (2001). Injury- and use-related plasticity in adult auditory cortex. *Audiol Neurootol*, *6*, 192-5.

Jacobson, G.P., Calder, J.A., Newman, C.W., Peterson, E.L., Wharton, J.A., & Ahmad, B.K. (1996). Electrophysiological indices of selective auditory attention in subjects with and without tinnitus. *Hear Res*, *97*, 66-74.

Jastreboff, P.J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci Res*, *8*, 221-54.

Jastreboff, P.J., Brennan, J.F., Coleman, J.K., & Sasaki, C.T. (1988). Phantom auditory sensation in rats: an animal model for tinnitus. *Behav Neurosci*, *102*, 811-22.

Jastreboff, P.J., & Jastreboff, M.M. (2000). Tinnitus Retraining Therapy (TRT) as a method for treatment of tinnitus and hyperacusis patients. *J Am Acad Audiol*, *11*, 162-77.

Jeanmonod, D., Magnin, M., & Morel, A. (1996). Low-threshold calcium spike bursts in the human thalamus. Common physiopathology for sensory, motor and limbic positive symptoms. *Brain*, *119 (Pt 2)*, 363-75.

Jemel, B., Achenbach, C., Muller, B.W., Ropcke, B., & Oades, R.D. (2002). Mismatch negativity results from bilateral asymmetric dipole sources in the frontal and temporal lobes. *Brain Topogr*, *15*, 13-27.

Kadner, A., Viirre, E., Wester, D.C., Walsh, S.F., Hestenes, J., Vankov, A., & Pineda, J.A. (2002). Lateral inhibition in the auditory cortex: an EEG index of tinnitus? *Neuroreport*, *13*, 443-6.

Kaltenbach, J.A., & Afman, C.E. (2000). Hyperactivity in the dorsal cochlear nucleus after intense sound exposure and its resemblance to tone-evoked activity: a physiological model for tinnitus. *Hear Res*, *140*, 165-72.

Kaltenbach, J.A., Zacharek, M.A., Zhang, J., & Frederick, S. (2004). Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. *Neurosci Lett*, *355*, 121-5.

Kamada, K., Moller, M., Saguer, M., Ganslandt, O., Kaltenhauser, M., Kober, H., & Vieth, J. (2001). A combined study of tumor-related brain lesions using MEG and proton MR spectroscopic imaging. *J Neurol Sci*, *186*, 13-21.

Kayser, J., Tenke, C., Nordby, H., Hammerborg, D., Hugdahl, K., & Erdmann, G. (1997). Event-related potential (ERP) asymmetries to emotional stimuli in a visual half-field paradigm. *Psychophysiology*, *34*, 414-26.

Kilgard, M.P., & Merzenich, M.M. (1998). Cortical map reorganization enabled by nucleus basalis activity. *Science*, *279*, 1714-8.

Komiya, H., & Eggermont, J.J. (2000). Spontaneous firing activity of cortical neurons in adult cats with reorganized tonotopic map following pure-tone trauma. *Acta Otolaryngol*, *120*, 750-6.

Kroener-Herwig, B., Biesinger, E., Gerhards, F., Goebel, G., Greinel, V., & Hiller, W. (2000). Retraining therapy for chronic tinnitus: a critical analysis of its status. *Scand Audiology*, *29*, 67-78.

Langguth, B., Eichhammer, P., Wiegand, R., Marienhegen, J., Maenner, P., Jacob, P., & Hajak, G. (2003). Neuronavigated rTMS in a patient with chronic tinnitus. Effects of 4 weeks treatment. *Neuroreport*, *14*, 977-80.

Lenarz, T. (1992). Epidemiologie. In H. Feldmann (Ed.), *Tinnitus* (pp. 71-75). Stuttgart: Thieme.

Llinas, R., Ribary, U., Contreras, D., & Pedroarena, C. (1998). The neuronal basis for consciousness. *Philos Trans R Soc Lond B Biol Sci*, *353*, 1841-9.

Llinas, R.R., Ribary, U., Jeanmonod, D., Kronberg, E., & Mitra, P.P. (1999). Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proc Natl Acad Sci U S A*, *96*, 15222-7.

Lockwood, A.H., Salvi, R.J., & Burkard, R.F. (2002). Tinnitus. *N Engl J Med*, *347*, 904-10.

Lockwood, A.H., Salvi, R.J., Coad, M.L., Arnold, S.A., Wack, D.S., Murphy, B.W., & Burkard, R.F. (1999). The functional anatomy of the normal human auditory system: responses to 0.5 and 4.0 kHz tones at varied intensities. *Cereb Cortex*, *9*, 65-76.

Lockwood, A.H., Salvi, R.J., Coad, M.L., Towsley, M.L., Wack, D.S., & Murphy, B.W. (1998). The functional neuroanatomy of tinnitus: evidence for limbic system links and neural plasticity. *Neurology*, *50*, 114-20.

Malenka, R.C., & Nicoll, R.A. (1999). Long-term potentiation--a decade of progress? *Science*, *285*, 1870-4.

McCarthy, G., Wood, C. (1985). Scalp distributions of event-related potentials: an ambiguity associated with analysis of variance models. *Electroencephalogr Clin Neurophysiol*, *62*, 203-208.

McDermott, H.J., Lech, M., Kornblum, M.S., & Irvine, D.R. (1998). Loudness perception and frequency discrimination in subjects with steeply sloping hearing loss: possible correlates of neural plasticity. *J Acoust Soc Am*, *104*, 2314-25.

McLean, J., & Palmer, L.A. (1998). Plasticity of neuronal response properties in adult cat striate cortex. *Vis Neurosci*, *15*, 177-96.

Meikle, M.B. (1997). Electronic access to tinnitus data: the Oregon Tinnitus Data Archive. *Otolaryngol Head Neck Surg*, *117*, 698-700.

Menning, H., Imaizumi, S., Zwitserlood, P., & Pantev, C. (2002). Plasticity of the human auditory cortex induced by discrimination learning of non-native, mora-timed contrasts of the Japanese language. *Learn Mem*, *9*, 253-67.

Menning, H., Roberts, L.E., & Pantev, C. (2000). Plastic changes in the auditory cortex induced by intensive frequency discrimination training. *Neuroreport*.

Merzenich, M.M., Kaas, J.H., Wall, J., Nelson, R.J., Sur, M., & Felleman, D. (1983). Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. *Neuroscience*, *8*, 33-55.

Merzenich, M.M., Nelson, R.J., Stryker, M.P., Cynader, M.S., Schoppmann, A., & Zook, J.M. (1984). Somatosensory cortical map changes following digit amputation in adult monkeys. *J Comp Neurol*, *224*, 591-605.

Mirz, F., Gjedde, A., Sodkilde-Jrgensen, H., & Pedersen, C.B. (2000). Functional brain imaging of tinnitus-like perception induced by aversive auditory stimuli. *Neuroreport*, *11*, 633-7.

Mirz, F., Pedersen, B., Ishizu, K., Johannsen, P., Ovesen, T., Stodkilde-Jorgensen, H., & Gjedde, A. (1999). Positron emission tomography of cortical centers of tinnitus. *Hear Res*, *134*, 133-44.

Møller, A.R. (1984). Pathophysiology of tinnitus. *Ann Otol Rhinol Laryngol*, *93*(1 Pt 1), 39-44.

Møller, A.R. (2003). Pathophysiology of tinnitus. *Otolaryngol Clin North Am*, *36*, 249-66, v-vi.

Moore, B.C. (1995). *Perceptual Consequences of Cochlear Damage*. Oxford: Oxford University Press.

Moore, B.C., & Alcantara, J.I. (2001). The use of psychophysical tuning curves to explore dead regions in the cochlea. *Ear Hear*, *22*, 268-78.

Moore, B.C., & Glasberg, B.R. (1983). Suggested formulae for calculating auditory-filter bandwidths and excitation patterns. *J Acoust Soc Am*, *74*, 750-3.

Moore, B.C., Huss, M., Vickers, D.A., Glasberg, B.R., & Alcantara, J.I. (2000). A test for the diagnosis of dead regions in the cochlea. *Br J Audiol*, *4*, 205-24.

Moore, B.C. (2003). *An introduction to the psychology of hearing*. (5 ed.). Amsterdam: Academic Press.

Moratti, S., Keil, A., & Stolarova, M. (2004). Motivated attention in emotional picture processing is reflected by activity modulation in cortical attention networks. *Neuroimage*, *21*, 954-64.

Mühlnickel, W., Elbert, T., Taub, E., & Flor, H. (1998). Reorganization of auditory cortex in tinnitus. *Proc Natl Acad Sci U S A*, *95*, 10340-3.

Muller, M., Klinke, R., Arnold, W., & Oestreicher, E. (2003). Auditory nerve fibre responses to salicylate revisited. *Hear Res*, *183*, 37-43.

Muller, M., Smolders, J.W., Ding-Pfennigdorff, D., & Klinke, R. (1997). Discharge properties of pigeon single auditory nerve fibers after recovery from severe acoustic trauma. *Int J Dev Neurosci*, *15*, 401-16.

Münte, T.F., Altenmüller, E., & Jäncke, L. (2002). The musician's brain as a model of neuroplasticity. *Nat Rev Neurosci*, *3*, 473-478.

Näätänen, R., & Alho, K. (1997). Mismatch negativity--the measure for central sound representation accuracy. *Audiol Neurootol*, *2*, 341-53.

Näätänen, R., & Escera, C. (2000). Mismatch negativity: clinical and other applications. *Audiol Neurootol*, *5*, 105-10.

Nakamura, K., Kawashima, R., Ito, K., Sugiura, M., Kato, T., Nakamura, A., Hatano, K., Nagumo, S., Kubota, K., Fukuda, H., & Kojima, S. (1999). Activation of the right inferior frontal cortex during assessment of facial emotion. *J Neurophysiol*, *82*, 1610-4.

Norena, A., Micheyl, C., & Chery-Croze, S. (2000). An auditory negative after-image as a human model of tinnitus. *Hear Res*, *149*, 24-32.

Norena, A., Micheyl, C., Chery-Croze, S., & Collet, L. (2002). Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol Neurootol*, *7*, 358-69.

Norena, A.J., & Eggermont, J.J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear Res*, *183*, 137-53.

Ochi, K., & Eggermont, J.J. (1997). Effects of quinine on neural activity in cat primary auditory cortex. *Hear Res*, *105*, 105-18.

Pantev, C., Oostenveld, R., Engelien, A., Ross, B., Roberts, L.E., & Hoke, M. (1998). Increased auditory cortical representation in musicians. *Nature*, *392*, 811-4.

Pantev, C., Weisz, N., Schulte, M., & Elbert, T. (2003). Plasticity of the human auditory cortex. In P. De Weerd (Ed.), *Filling-In: From perceptual completion to cortical reorganization*. (pp. 231-251). Oxford: Oxford University Press.

Pantev, C., Wollbrink, A., Roberts, L.E., Engelien, A., & Lutkenhoner, B. (1999). Short-term plasticity of the human auditory cortex. *Brain Res*, *842*, 192-9.

Penner, M.J. (1983). Variability in matches to subjective tinnitus. *J Speech Hear Res*, *26*, 263-7.

Pessoa, L., & De Weerd, P. (Eds.). (2003). *Filling-in: from perceptual completion to cortical reorganization*. Oxford: Oxford University Press.

Pilgramm, M., Rychlik , R., Lebisch, H., Siedentop, H., Goebel, G., & Kirchhoff, D. (1999). Tinnitus in der Bundesrepublik Deutschland - eine repräsentative epidemiologische Studie. *HNO aktuell*, *7*, 261-265.

Pleger, B., Dinse, H.R., Ragert, P., Schwenkreis, P., Malin, J.P., & Tegenthoff, M. (2001). Shifts in cortical representations predict human discrimination improvement. *Proc Natl Acad Sci U S A*, *98*, 12255-60.

Plewnia, C., Bartels, M., & Gerloff, C. (2003). Transient suppression of tinnitus by transcranial magnetic stimulation. *Ann Neurol*, *53*, 263-6.

Pons, T.P., Garraghty, P.E., Ommaya, A.K., Kaas, J.H., Taub, E., & Mishkin, M. (1991). Massive cortical reorganization after sensory deafferentation in adult macaques. *Science*, *252*, 1857-60.

Rachel, J.D., Kaltenbach, J.A., & Janisse, J. (2002). Increases in spontaneous neural activity in the hamster dorsal cochlear nucleus following cisplatin treatment: a possible basis for cisplatin-induced tinnitus. *Hear Res*, *164*, 206-14.

Rajan, R. (1998). Receptor organ damage causes loss of cortical surround inhibition without topographic map plasticity. *Nat Neurosci*, *1*, 138-43.

Rajan, R., & Irvine, D.R. (1998). Neuronal responses across cortical field A1 in plasticity induced by peripheral auditory organ damage. *Audiol Neurootol*, *3*, 123-44.

Rajan, R., Irvine, D.R., Wise, L.Z., & Heil, P. (1993). Effect of unilateral partial cochlear lesions in adult cats on the representation of lesioned and unlesioned cochleas in primary auditory cortex. *J Comp Neurol*, *338*, 17-49.

Ramachandran, V.S. (1993). Behavioral and magnetoencephalographic correlates of plasticity in the adult human brain. *Proc Natl Acad Sci U S A*, *90*, 10413-20.

Rauschecker, J.P. (1999). Auditory cortical plasticity: a comparison with other sensory systems. *Trends Neurosci*, *22*, 74-80.

Recanzone, G.H., Merzenich, M.M., Jenkins, W.M., Grajski, K.A., & Dinse, H.R. (1992). Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. *J Neurophysiol*, *67*, 1031-56.

Recanzone, G.H., Schreiner, C.E., & Merzenich, M.M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci*, *13*, 87-103.

Reyes, S., Salvi, R., Burkard, R.F., Coad, M.L., Wack, D., Galantowicz, P., & Lockwood, A. (2002). Brain imaging of the effects of lidocaine on tinnitus. *Hear Res*, *171*, 43-50.

Röder, B., Teder-Salejarvi, W., Sterr, A., Rösler, F., Hillyard, S.A., & Neville, H.J. (1999). Improved auditory spatial tuning in blind humans. *Nature*, *400*, 162-6.

Romanski, L.M., & Goldman-Rakic, P.S. (2002). An auditory domain in primate prefrontal cortex. *Nat Neurosci*, *5*, 15-6.

Ross, B., Borgmann, C., Draganova, R., Roberts, L.E., & Pantev, C. (2000). A high-precision magnetoencephalographic study of human auditory steady-state responses to amplitude-modulated tones. *J Acoust Soc Am*, *108*, 679-91.

Salvi, R.J., Hamernik, R.P., & Henderson, D. (1983). Response patterns of auditory nerve fibers during temporary threshold shift. *Hear Res*, *10*, 37-67.

Salvi, R.J., Wang, J., & Ding, D. (2000). Auditory plasticity and hyperactivity following cochlear damage. *Hear Res*, *147*, 261-74.

Scherg, M. (1991). *Akustisch evozierte Potentiale: Grundlagen - Entstehungsmechanismen - Quellenmodell*. Stuttgart: Kohlhammer.

Scherg, M., & Von Cramon, D. (1986). Evoked dipole source potentials of the human auditory cortex. *Electroencephalogr Clin Neurophysiol*, *65*, 344-60.

Schneider, P., Scherg, M., Dosch, H.G., Specht, H.J., Gutschalk, A., & Rupp, A. (2002). Morphology of Heschl's gyrus reflects enhanced activation in the auditory cortex of musicians. *Nat Neurosci*, *7*, 688-694.

Schwaber, M.K., Garraghty, P.E., & Kaas, J.H. (1993). Neuroplasticity of the adult primate auditory cortex following cochlear hearing loss. *Am J Otol*, *14*, 252-8.

Shiomi, Y., Tsuji, J., Naito, Y., Fujiki, N., & Yamamoto, N. (1997). Characteristics of DPOAE audiogram in tinnitus patients. *Hear Res*, *108*, 83-8.

Steriade, M. (1993). Central core modulation of spontaneous oscillations and sensory transmission in thalamocortical systems. *Curr Opin Neurobiol*, *3*, 619-25.

Sterr, A., Müller, M.M., Elbert, T., Rockstroh, B., Pantev, C., & Taub, E. (1998). Perceptual correlates of changes in cortical representation of fingers in blind multifinger Braille readers. *J Neurosci*, *18*, 4417-23.

Stouffer, J.L., & Tyler, R.S. (1990). Characterization of tinnitus by tinnitus patients. *J Speech Hear Disord*, *55*, 439-53.

Syka, J. (2002). Plastic changes in the central auditory system after hearing loss, restoration of function, and during learning. *Physiol Rev*, *82*, 601-36.

Taub, E., Uswatte, G., & Elbert, T. (2002). New treatments in neurorehabilitation founded on basic research. *Nat Rev Neurosci*, *3*, 228-236.

Thai-Van, H., Micheyl, C., Moore, B.C., & Collet, L. (2003). Enhanced frequency discrimination near the hearing loss cut-off: a consequence of central auditory plasticity induced by cochlear damage? *Brain*, *126*(Pt 10), 2235-45.

Thai-Van, H., Micheyl, C., Norena, A., & Collet, L. (2002). Local improvement in auditory frequency discrimination is associated with hearing-loss slope in subjects with cochlear damage. *Brain*, *125*(Pt 3), 524-37.

Uchida, S., Maloney, T., March, J.D., Azari, R., & Feinberg, I. (1991). Sigma (12-15 Hz) and delta (0.3-3 Hz) EEG oscillate reciprocally within NREM sleep. *Brain Res Bull*, *27*, 93-6.

Vieth, J.B., Kober, H., & Grummich, P. (1996). Sources of spontaneous slow waves associated with brain lesions, localized by using the MEG. *Brain Topogr*, *8*, 215-21.

Wallhäusser-Franke, E., Braun, S., & Langner, G. (1996). Salicylate alters 2-DG uptake in the auditory system: a model for tinnitus? *Neuroreport*, *7*, 1585-8.

Wallhäusser-Franke, E., Mahlke, C., Oliva, R., Braun, S., Wenz, G., & Langner, G. (2003). Expression of c-fos in auditory and non-auditory brain regions of the gerbil after manipulations that induce tinnitus. *Exp Brain Res*, *153*, 649-54.

Walpurger, V., Hebing-Lennartz, G., Denecke, H., & Pietrowsky, R. (2003). Habituation deficit in auditory event-related potentials in tinnitus complainers. *Hear Res*, *181*, 57-64.

Weinberger, N.M. (1998). Physiological memory in primary auditory cortex: characteristics and mechanisms. *Neurobiol Learn Mem*, *70*, 226-51.

Weisz, N., Voss, S., Berg, P., & Elbert, T. (2004a). Abnormal auditory mismatch response in tinnitus sufferers with high-frequency hearing loss is associated with subjective distress level. *BMC Neuroscience*, 5:8.

Weisz, N., Keil, A., Wienbruch, C., Hoffmeister, S., & Elbert, T. (2004b). One set of sounds, two tonotopic maps – exploring auditory cortex with amplitude-modulated tones. *Clinical Neurophysiology, 115*, 1248-1258.

Weisz, N., Wienbruch, C., Hoffmeister, S., & Elbert, T. (2004c). Tonotopic organization of the human auditory cortex probed with frequency-modulated tones. *Hearing Research, 191*, 49-58.

Wheeler, R.E., Davidson, R.J., & Tomarken, A.J. (1993). Frontal brain asymmetry and emotional reactivity: a biological substrate of affective style. *Psychophysiology*, *30*, 82-9.

Wienbruch, C., Moratti, S., Elbert, T., Vogel, U., Fehr, T., Kissler, J., Schiller, A., & Rockstroh, B. (2003). Source distribution of neuromagnetic slow wave activity in schizophrenic and depressive patients. *Clin Neurophysiol*, *114*, 2052-60.

Willott, J.F. (1996). Physiological plasticity in the auditory system and its possible relevance to hearing aid use, deprivation effects, and acclimatization. *Ear Hear*, *17*(3 Suppl), 66S-77S.

Willott, J.F., & Carlson, S. (1995). Modification of the acoustic startle response in hearing-impaired C57BL/6J mice: prepulse augmentation and prolongation of prepulse inhibition. *Behav Neurosci*, *109*(3), 396-403.

Willott, J.F., Carlson, S., & Chen, H. (1994). Prepulse inhibition of the startle response in mice: relationship to hearing loss and auditory system plasticity. *Behav Neurosci*, *108*, 703-13.

Appendix A

Manuscript of:

One set of sounds, two tonotopic maps - exploring auditory cortex with amplitude-modulated tones

Weisz, N., Keil, A., Wienbruch, C., Hoffmeister, S. & Elbert, T.

Submitted to *Clinical Neurophysiology*
Accepted for publication: 31.12.2003
Current status: in press

Abstract

The possibility to simultaneously observe activation of primary and secondary auditory cortices has been demonstrated by Engelien et al. (2000). Such a dual monitoring by means of neuromagnetic recordings can be achieved when a subject is stimulated by brief pulses of 40-Hz-modulated tones. Depending on the frequency filter applied, either the steady-state field (SSF) or the N1m can be extracted from the evoked magnetic field complex. Using this "combined" (two-maps) paradigm with four carrier frequencies, we show that it is possible to synchronously screen two tonotopic maps – one map each reflected either by the SSF or the N1m. Indicators are the systematic variation in the location (higher frequencies are more posterior) and orientation (higher frequencies oriented differently in the saggital plane) of the equivalent current dipole (ECD). These parameters were compared with those obtained from "classic" (one map) paradigms in which either a pure tone elicits a N1m or a 40-Hz continuous (3 seconds) stimulation produces a SSF. Overall the results were similar, however systematic differences between the paradigms were found for ECD localization, dipole strength, amplitude, and phase. One possible interpretation of these results is that different tonotopically arranged cortical fields were involved in the generation of the components.

Keywords: Tonotopy, N1m, Steady-State Field, Magnetencephalography, Magnetic source imaging

Introduction

From both animal (Merzenich et al., 1975, Kaas et al., 1999) and human studies (Romani et al., 1982; Romani et al., 1986; Pantev et al., 1993; Pantev et al., 1996) it is well-known that neurons in the auditory cortex are arranged tonotopically (i.e. frequency specific), thus resembling the spatial order of the inner hair cells on the basilar membrane. However, speaking of *the* tonotopic organization of the auditory cortex can be misleading. The auditory cortex can be divided into several areas, each comprising multiple subareas. Each subarea exhibits tonotopy to a varying degree and manner (Rouiller,1997). According to Kaas et al. (1999) the main hierarchically organized areas in primates are the core (including the primary auditory field, AI), the belt and the parabelt, out of which the core can be considered the actual primary auditory cortex. The different areas of the core show the highest tonotopic order with an either anterior/anterolateral to posterior/posteromedial or directly opposite gradient depending on the field (see also Ehret, 1997).

So far, magnetencephalography (MEG) has proven to be a successful non-invasive technique to study the tonotopic organization of the auditory cortex in humans. The standard MEG approach to investigate tonotopy consists in source localization of the N1m (operationalized as a single equivalent current dipole in a homogeneous sphere) elicited by pure tones of a few hundred milliseconds duration with varying frequency. Although, the first study (Romani et al., 1982) to non-invasively demonstrate tonotopy in man used steady-state stimuli, such attempts have been scarce (Pantev et al., 1996). Typical auditory steady-state stimuli are amplitude or frequency modulated tones. Initial perturbations of the signal can be discarded if the stimuli are sufficiently long (seconds to minutes). Typically a modulation rate of 35-40 Hz is chosen in order to obtain maximum response energy (Galambos et al., 1981; Hari et al., 1989; Pastor et al., 2002; Roß et al., 2000).

In the following, we will refer to these two conventional approaches (i.e. elicitation of the N1m by pure tones and the SSF by continuous 40-Hz modulated tones) as *"classic" paradigms*. They are differentiated from a *"combined" paradigm*, a term introduced by Engelien et al. (2000). It refers to the possibility of extracting the transient N1m- and the steady-state response to the same stimulus, which has been first shown by Mäkelä and Hari (1987). A simultaneous – and thus time-economic – measurement of both components is desirable, since sources for these components are

associated with activations from primary or secondary auditory cortex respectively (see below). Engelien et al. raised the question whether the N1m and SSF gained at the same time via the 'combined' approach would lead to identical source localizations as if assessed by the 'classic' approach. These authors presented a pure tone with a carrier frequency of 250 Hz and a duration of 500 ms for elicitation of the N1m, and a continuous 200 s 39-Hz amplitude modulated tone was used for elicitation of the SSF, thus representing 'classic' paradigms. A third – "combined" – paradigm presented the same carrier frequency as 500 ms 39-Hz amplitude modulated tones, thus combining features of both aforementioned paradigms, in order to gain the N1m and SSF simultaneously. The authors demonstrated that the components obtained simultaneously and their classical counterparts a) showed the same pattern of differences and b) seemed not to differ in location. However, only one single carrier frequency was used, i.e., no information was obtained with respect to tonotopy. The main goal of the present study was to see if and which tonotopic *maps* can be obtained by using the "combined" paradigm described above on four different carrier frequencies.

In the past, three neuromagnetic measures served as indicators for the presence of tonotopically arranged neuronal populations ("fields") in the auditory cortex in humans: a) change of the location of a component as a function of the frequency of the eliciting stimulus b) spatial separation of tonotopic gradient in dependence of the component investigated and c) orientation of the dipole.

Direction of tonotopic gradient: For the N1m some authors have found a low-to-high frequency specific arrangement of ECD sources on the anterior-posterior axis (Elberling et al., 1982). However the great majority of MEG studies localize the main tonotopic gradient on a medial-lateral axis (Lütkenhöner and Steinsträter, 1998; Pantev et al., 1993; Elbert et al., 2002) with the depth of the source linearly increasing with the logarithm of the carrier frequency. Those studies investigating SSF have also reported tonotopy for high-to-low frequencies in the medial-lateral direction (Romani et al., 1982, Pantev et al., 1996, Roß et al., 2000). While the SSF map seems consistent with primate studies (Kaas et al, 1999), BOLD imaging in humans confirms the significance of the spatial gradient for the N1m showing that the center of activation shifts in a posterior and medial direction for higher frequencies (Lauter et al., 1985; Wessinger et al., 2001).

Spatial separation of tonotopic gradient in dependence of the component: Although the direction of the tonotopic gradient seems to be the same for the N1m and SSF, ECD sources have significantly different locations, thus suggesting that different areas of the auditory cortex may be activated. In comparison to the N1m, which has been associated with activation of the secondary auditory cortex and lateral parts of Heschl's gyrus (Lütkenhöner and Steinsträter, 1998; Godey et al., 2001), sources for SSF seem to be located more anterior and more medial and have been related to activation of the primary auditory cortex (Engelien et al., 2000; Pantev et al., 1996; Tiihonen et al., 1989). Overall, data from neuromagnetic studies confirm that the human auditory cortex – like the one in primates – can be subdivided into different tonotopically arranged fields. This view is supported by recent fMRI (Talavage et al, 2000) and post-mortem histochemical studies (Wallace et al., 2002). However, the exact number of different fields in human auditory cortex remains a matter of controversy (Talavage et al, 2000).

Dipole orientation in the saggital plane: For the N1m, some authors reported a systematic relationship between frequency and dipole orientation (Tiitinen et al., 1993; Verkindt et al., 1995). Generally, when viewed from the lateral surface in the saggital plane, the angle in the right hemisphere rotates clockwise with increasing frequency of the stimulus. According to Verkindt et al. (1995) this might be due to gyral folding characteristics varying with depth. Tiitinen et al. (1993) were able to show the same pattern for the Mismatch Negativity. To the best of our knowledge, no work has yet investigated whether such an association between dipole orientation and frequency also exists for the SSF.

According to the criteria mentioned above dipole localizations on two of the three coordinate axes (posterior-anterior, medial-lateral) and dipole orientation in the saggital plane served as indicators of tonotopy. Sources of the N1m and SSF were regarded as activations from different auditory fields if they were separable in space. The results from the combined paradigm were compared to results obtained with the classic paradigms to elicit a N1m and a SSF. We compared the following dependent variables: localization on the three coordinate axes, dipole orientation, dipole strength (Q), peak amplitude and peak latency / phase (in case of the N1m or SSF respectively).

Methods

Subjects

Eleven right-handed participants (6 females; age range: 20-28 years) without neurological or otological disorders gave informed consent after obtaining written and oral information on the nature of the study. They received € 15 for participation.

Neuromagnetic recording

Neuromagnetic data were recorded (A/D conversion rate: 678.17 Hz; 0.1-200 Hz bandpass) with a 148 channel whole-head magnetometer (4D Neuroimaging Inc. , San Diego). Vertical and horizontal electro-oculogram (EOG) were measured from above and below the eye and from the outer canthi in order to reject epochs contaminated by eye-movements and eye-blinks. These were identified by amplitudes exceeding 100 μV.

Procedurce

Auditory stimuli were monaurally presented via a plastic tube attached to an earpiece in the left ear of the participant. Individual sensation levels (SL) were assessed prior to the MEG-measurement for each frequency-stimulus type combination. Sounds were presented at an intensity of 50 dB SL. During the neuromagnetic recording session, subjects laid in a supine position watching a film of their choice which was projected to the ceiling of the magnetically shielded room.

We used four carrier frequencies of 1000, 1817, 3302 and 6000 Hz which were presented blockwise in three different manners:

N1m - classic paradigm: Pure tones of 500 ms duration (5 ms rise and fall time; 200 stimuli; SOA varying between 1500-2500 ms); component of interest: N1m; presumed activation from secondary auditory cortex.

SSF-classic paradigm: Amplitude modulated tones of 3 s duration (50 stimuli; SOA varying between 4000-4500 ms). Modulation rate was 39-Hz, modulation depth was 100%; component of interest: SSF; presumed activation from primary auditory cortex.

Combined paradigm: Analoguous to SSF-classic, except that duration was shortened to 500 ms (200 stimuli; SOA varying between 1500-2500 ms). The N1m *and* the SSF can be extracted from the compound evoked magnetic field by different digital band pass filters; presumed activations from primary and secondary auditory cortex.

Data Analysis

N1m was analyzed in the N1m-classic and the bi-mapping conditions. For this purpose a time-window ranging from 150 ms before and 300 ms after stimulus onset was selected. Artefact free epochs were averaged and filtered with a 1-20 Hz digital bandpass (Butterworth characteristic of 2nd order). For each subject a group of 38 magnetic sensors was determined over the right hemisphere that both, covered well the evoked magnetic fields and could best explain the observed magnetic fields. Magnetic source imaging (MSI) was performed for each of the 12 runs. We used a homogeneous sphere as volume conductor, which fitted best the subjects head underneath the selected sensor in a least square sense. Single equivalent current dipoles (ECD) were fitted for each sampling point 10 ms before and 4 ms after the peak of the N1m. A median was calculated from those resulting sources with a minimum goodness of fit (GOF) of .90 or better.

The SSF-classic and the bi-mapping paradigm were designed to produce a 39-Hz oscillatory SSF. Hence data were filtered with a 37-41 Hz digital bandpass (Butterworth characteristic 2nd order) and split into epochs of 102 ms following stimulus onset. The first and last epoch of the stimulus, i.e. from 0 ms - 102 ms after stimulus onset and 102 ms before stimulus offset were excluded from further analysis. Thus approximately 1370 (in the case of SSF-classic) or 580 epochs (in the case of SSF-combined) entered the averaging procedure for the respective paradigm. As for the N1m-analysis a sensor group was determined for every subject. The resulting ECD sources for every time point were arranged according to their GOF and the best three fits were accepted if their localizations met following criteria: a posterior-anterior range from 0 to 4 cm and medial-lateral not more positive (meaning further towards left) than –2 cm. These additional criteria were included, as the GOF for the SSF (M = .87, SD = .05) was approximately 10% lower than the one for the N1m (M = .97, SD = .01), independent of paradigm or carrier frequency.

As indicators of tonotopy we chose source localization on the medial-lateral (y-axis) and posterior-anterior (x-axis). Furthermore dipole orientation in the saggital plane was investigated using following formula:

$angle(degrees) = atan(Q_z/Q_x)/p*180$,

where Q_z and $Q_x$are the projections of the dipole moment in the inferior-superior (z-) and posterior-anterior (x-) direction respectively. The major interest was to see whether two tonotopic maps could be obtained simultaneously. Another goal was to determine whether the different approaches (i.e., classic vs. combined) for the components would produce similar results. Comparisons were made in terms of the ECD results (position, strength, orientation) but also on measures of the waveform (amplitude and latency / phase). Statistical analysis was accomplished with analysis of variance (ANOVA) for repeated measurements. In the case of significant results ($p < .05$) of the ANOVA the Tukey-Kramer procedure was applied for post-hoc analysis. In order to analyze the 39-Hz steady-state signal in terms of amplitude and phase however a different approach was chosen as the knowledge about the specific frequency content can be employed. Averaged data were first projected into source space (197 sources) by means of the minimum-norm estimate (MNE), a linear estimation technique first introduced Hämäläinen and Ilmoniemi (1984). 197 dipole locations, evenly spaced on a spherical shell (60% of radius of the fitted sphere), were used as source space (Hauk et al., 2002). Each location consisted of two perpendicular dipoles oriented tangentially to the shell surface. An FFT was then performed over the entire source space activity. The resulting complex values for the 39-Hz activity of every participant were sorted into different arrays according to the conditions. These arrays were submitted to circular T^2 algorithm (Victor and Mast, 1991), which is sensitive to differences between samples of complex Fourier values reflecting oscillatory activity in different experimental conditions. To illustrate the contributions of 39 Hz FFT amplitude and phase to the T-square effects observed, we used a planned comparisons approach. Previous studies (e.g. Roß et al., 2000) demonstrated a negative relationship between carrier frequency of the stimulus and amplitude or phase of the auditory SSF. Therefore we reduced the 24 difference maps reflecting all possible pairwise comparisons to four maps (2 paradigms x 2 dependent variables). To this end we used linear contrasts for repeated measurements (Rosenthal and Rosnow, 1985). These were computed at each dipole location of L2-norm solution space, delineating the statistical significance and effect size of the hypothesized relationship stated above, namely a linear decrease of phase and amplitude as a function of carrier frequency. More details are given in the results section.

Results

N1m peak latency

From N1m waveforms depicted in Figure 1a it can be seen that the N1m deflections peaked at approximately 100 ms. However, for the combined paradigm they showed a consistent delay of about 7 ms for all the frequencies (M = 104.54 ms, SE = 1.62) compared to their counterparts derived from the classic paradigm (M = 97.47 ms, SE = 1.64; $F_{1,10} = 46.66$, $p < .001$). A significant effect on latency was found for the

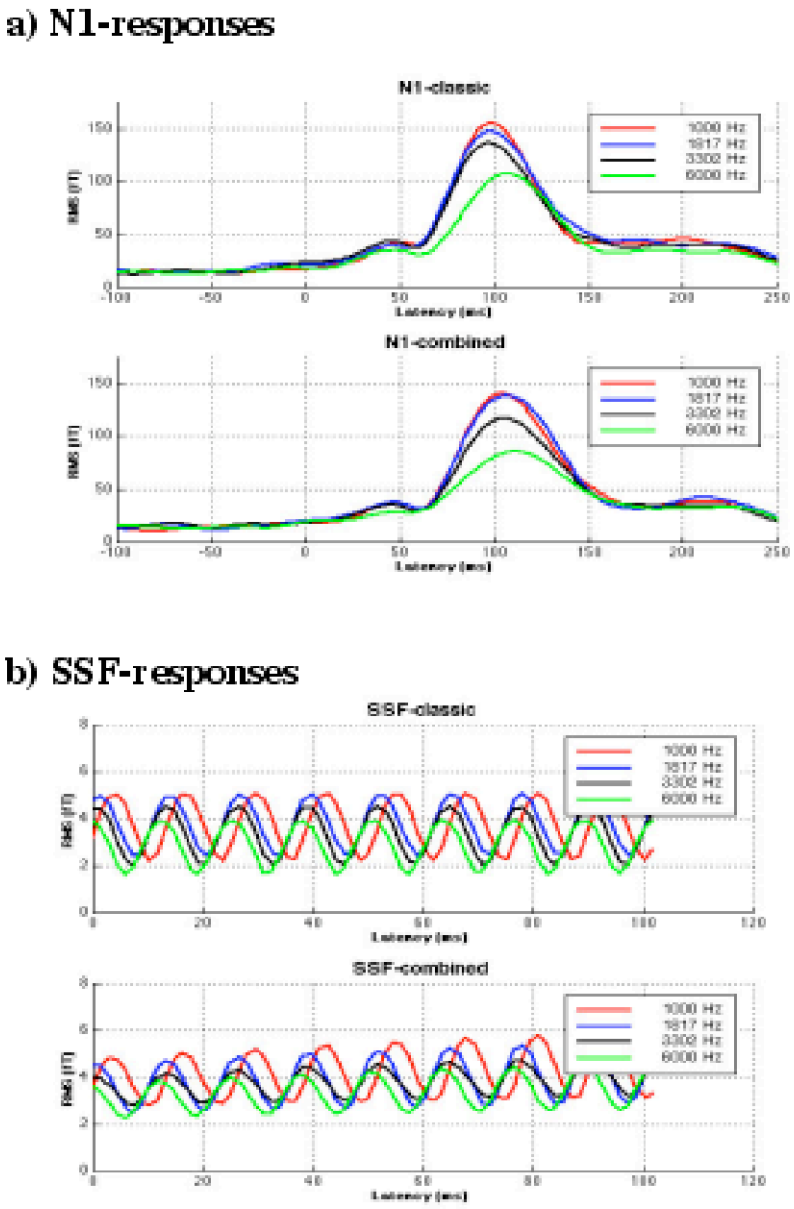

Figure 1: Grand average waveforms in the signal space for the N1m (a) and SSF (b), derived from the RMS of a 38 sensor group centered over the Sylvian fissure of the right hemisphere.

factor frequency ($F_{3,30} = 3.89$, $p < .02$). This effect was due to a later N1m response for the 6000 Hz stimulus, the difference ranging between 6 (compared to 1000 Hz) and 8 ms (compared to 1817 and 3302 Hz).

N1m amplitude

Marked differences between the paradigms were also obtained for the amplitude of N1m (see Figure 1a), which was about 15-20 fT smaller for signals of the bi-mapping ($M = 128.21$ fT, $SE = 7.05$) than for the classic paradigm ($M = 143.55$ fT, $SE = 7.32$) independent of frequency ($F_{1,10} = 7.51$, $p < .03$). Concerning frequency, the 1000 Hz stimuli yielded the strongest response ($M = 156.95$ fT, $SE = 10.98$) and 6000 Hz ($M = 102.36$ fT, $SE = 6.90$) the weakest, the amplitudes of the other two frequencies lying in between (1817 Hz: $M = 150.93$ fT; 3302 Hz: $M = 133.29$ fT). The relative weakness of the response to the 6000 Hz stimuli led to a significant ANOVA effect of frequency ($F_{3,30} = 13.64$, $p < .001$).

SSF phase and amplitude

The circular T^2 statistic revealed strong and widespread differences with a focus over the right hemisphere regardless of the paradigm. Figure 2a shows the most pronounced difference, which was obtained for the comparison between 1000 and 6000 Hz. Since an a priori hypothesis existed, linear contrast analysis statistics were applied for further analysis (Rosenthal and Rosnow, 1985). This method yields an F- (here: critical $F_{1,33}$ at .05 significance level: 4.14) and an effect-size value $r_{contrast}$ (contrast correlations ranging from -1 to 1; positive values showing a trend in the predicted direction). Following lambda-weights for the four conditions were chosen: -3 (6000 Hz), -1 (3302 Hz), 1 (1817 Hz) and 3 (1000 Hz) indicating a linear decrease in amplitude and a phase advance with increasing carrier frequency (Roß et al., 2000). A clear linear trend for the predicted direction (both paradigms) could be observed contralateral to stimulation (Figure 2b and c). Thus, frequency effects appear to be reflected in changes of phase and amplitude, which is in line with inspection of the time-domain waveforms (see Figure 1b). Looking at the F- and $r_{contrast}$-values, the trend was more pronounced for the classic condition, especially with respect to amplitude.

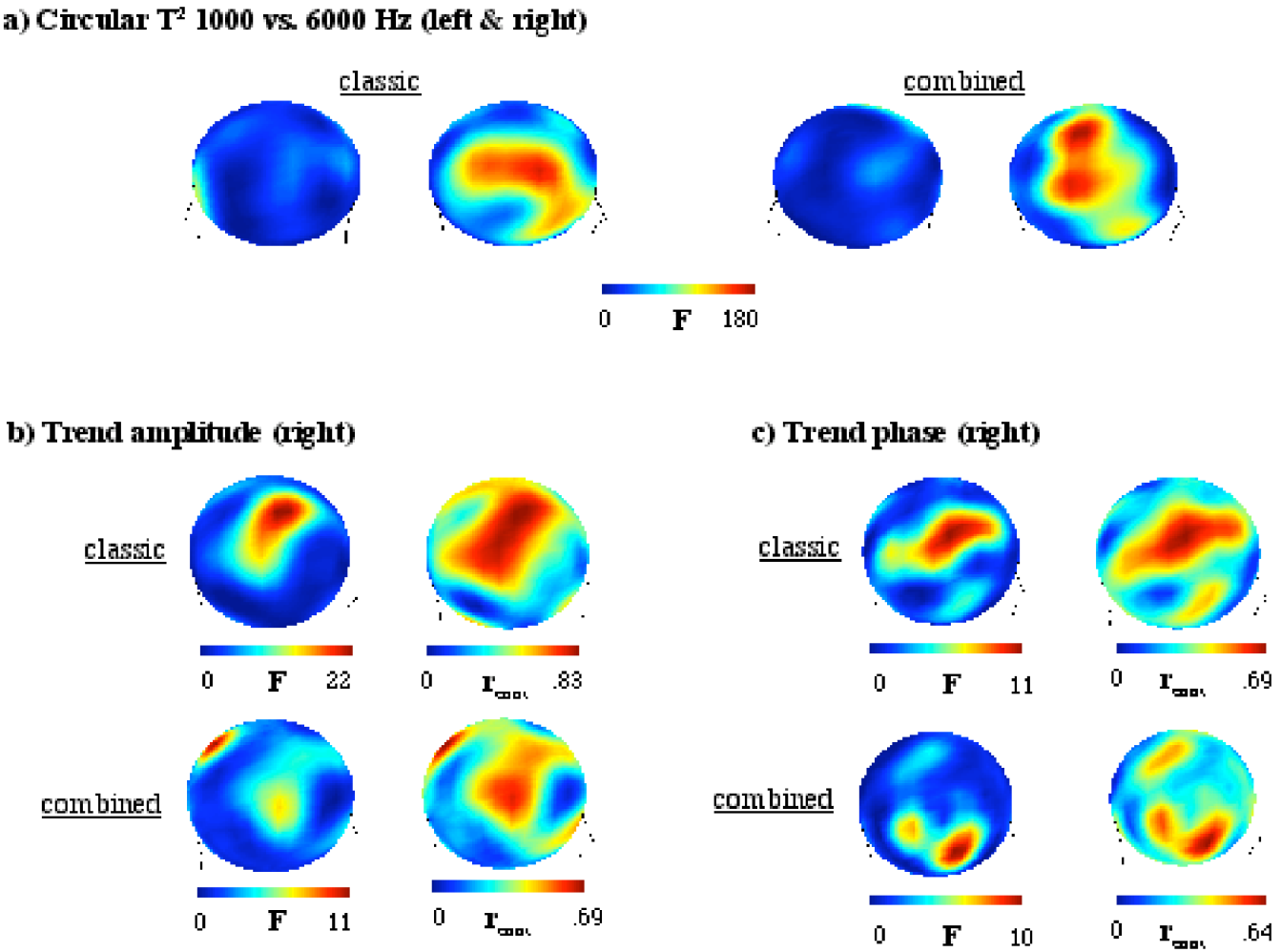

Figure 2: Statistical analysis of source space projected frequency domain results (pairwise comparisons using the circular T^2 statistic). a) shows F-values for the comparison between the 1000 and 6000 Hz condition for both paradigms. Multiple comparisons could be summarized using linear contrast analysis yielding a F- and effect size map for b) amplitude and c) phase.

Dipole moment

Similar to other results reported here, the 6000 Hz condition showed a pronounced deviance from the other conditions in terms of dipole moment. On this measure (see Table 1) source strengths evoked by a 6000 Hz stimulus were smaller compared to the strength of sources evoked by stimuli of other frequencies ($F_{3,30}$ = 3.85, p < .02). However the difference reached statistical significance only for the comparison 1000 vs. 6000 Hz of the N1m, thus resulting in a frequency x component interaction ($F_{3,30}$ = 3.26, p < .04). Moreover, dipole moments of the combined condition were smaller than those of the classical condition ($F_{1,10}$ = 6.91, p < .03). A closer inspection showed a reversed pattern of the dipole moment evoked by the two paradigms depending on the component. Post-hoc analysis showed that the paradigm x component interaction ($F_{1,10}$ = 8.96, p < .02) is due to the significantly stronger source strength of the classical paradigm in the N1m condition.

Table 1: Average dipole strength (Q) for all *frequency x component x paradigm* conditions.

Frequency (Hz)	component	Paradigm	*Mean (nAm)*	*SE*
1000	N1	classic	28.28	2.61
		Combined	25.47	3.53
	SSF	classic	1.02	0.14
		Combined	1.63	0.50
1817	N1	classic	28.55	3.46
		Combined	24.97	3.26
	SSF	classic	0.91	0.12
		Combined	0.94	0.10
3302	N1	classic	27.75	3.22
		Combined	25.16	4.12
	SSF	classic	1.09	0.15
		Combined	1.20	0.13
6000	N1	classic	22.93	2.96
		Combined	16.13	1.43
	SSF	classic	0.61	0.08
		Combined	0.80	0.14

Source localizations

All results of ECD analysis are depicted in Table 2. Interestingly, a tonotopic gradient was most pronounced on the anterior-posterior axis with higher frequencies being located further towards posterior locations ($F_{3,30}$ = 5.82, p < .003). Post-hoc analyses indicate that sources evoked by 6000 Hz stimuli were significantly more posteriorally located than sources to 1000 and 1817 Hz. The comparisons 1000 vs. 3302 Hz (mean

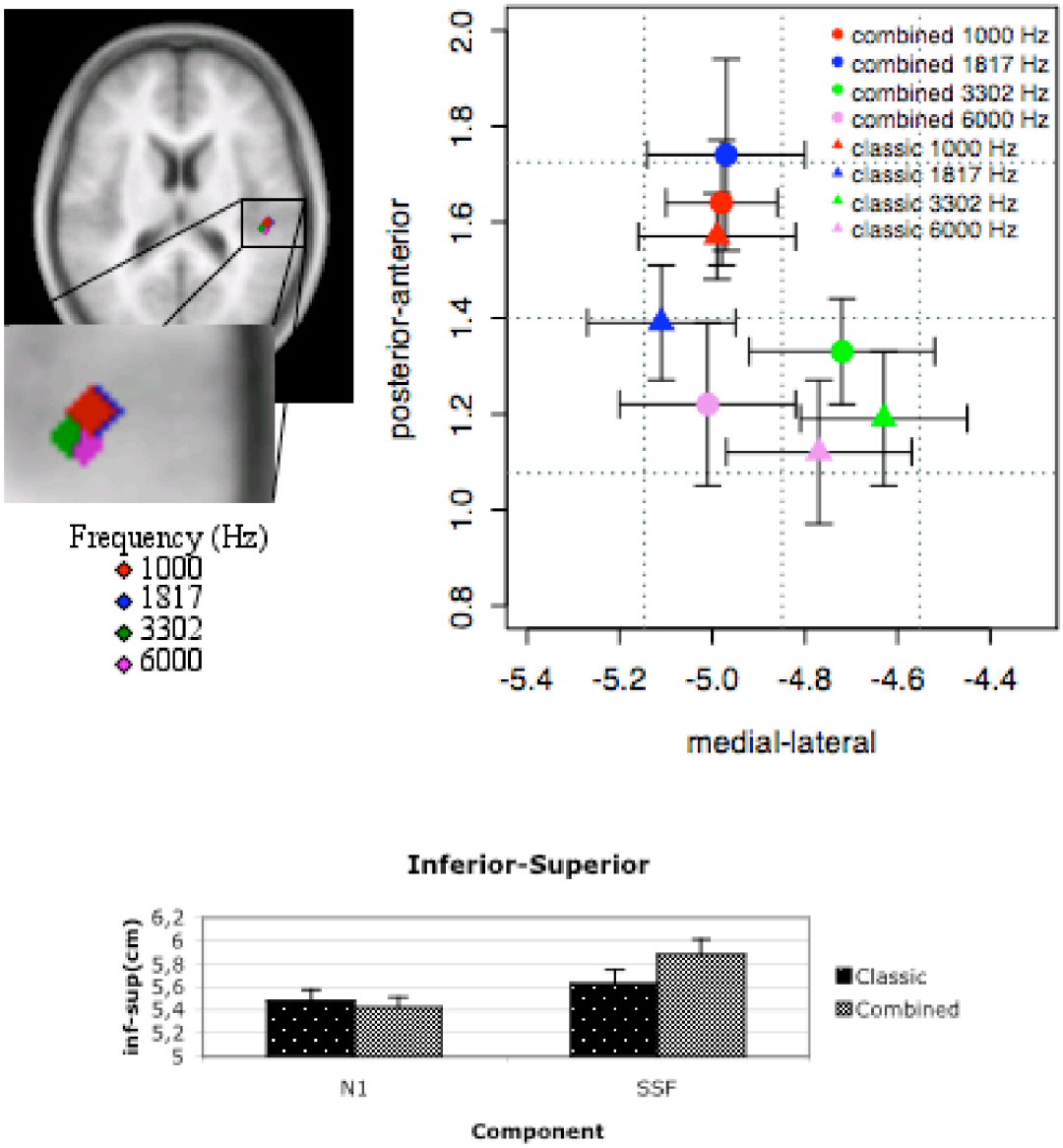

Figure 3: The upper panel shows source localization on an axial plane. Source localization for the different frequencies is displayed on a standard brain (left upper panel). They are plotted (+/- SE) for the two paradigms separately in the right upper panel (note that more negative values on the x-axis indicates locations further to the right). The lower panel displays the location for the components in the inferior-superior direction separately for the two paradigms.

difference: .34 cm) and 1817 vs. 3302 Hz (mean difference: .31 cm) approached statistical significance (critical difference: .34 cm). Concerning the paradigms it can be taken from Table 2 that sources were – with the exception of the SSF elicited by the 6000 Hz amplitude modulated tone – consistently more anterior than those from the classical paradigm ($F_{1,10} = 11.20$, $p < .01$). Neither the main effect for the factor *component* nor any interactions were significant (all $F < 1$), Figure 3 (left upper

Table 2:ECD localizations for all *frequency x component x paradigm* combinations. Note that a more negative values mean further towards the right.

Frequency (Hz)	component	Paradigm	Posterior-anterior (*x*)		medial-lateral (*y*)		inferior-superior (*z*)	
			Mean (cm)	*SE*	*Mean (cm)*	*SE*	*Mean (cm*	*SE*
1000	N1	Classic	1.51	0.12	-5.24	0.24	5.50	0.19
		Combined	1.54	0.15	-5.35	0.15	5.53	0.14
	SSF	Classic	1.63	0.15	-4.75	0.29	5.84	0.23
		Combined	1.74	0.23	-4.62	0.12	5.96	0.34
1817	N1	Classic	1.38	0.13	-5.29	0.24	5.36	0.19
		Combined	1.62	0.16	-5.34	0.16	5.42	0.19
	SSF	Classic	1.42	0.23	-4.93	0.21	5.72	0.12
		Combined	1.86	0.38	-4.60	0.26	5.95	0.24
3302	N1	Classic	1.22	0.12	-5.10	0.22	5.51	0.15
		Combined	1.41	0.16	-5.03	0.31	5.28	0.18
	SSF	Classic	1.17	0.27	-4.17	0.22	5.55	0.21
		Combined	1.26	0.16	-4.42	0.24	5.93	0.16
6000	N1	Classic	1.09	0.13	-4.87	0.29	5.55	0.24
		Combined	1.38	0.16	-5.24	0.21	5.46	0.17
	SSF	Classic	1.17	0.29	-4.68	0.31	5.42	0.32
		Combined	1.08	0.30	-4.80	0.32	5.69	0.26

panel) shows the localizations collapsed across components overlayed on a standard brain.

In contrast, a tonotopic gradient was not observable for the medial-lateral axes (Figure 3 upper panel), despite a significant effect for frequency ($F_{3,30} = 3.19$, $p < .05$). As a post-hoc analysis however revealed, this effect was due to a significantly more medial localization of sources evoked during 3302 Hz stimuli in comparison to 1817 Hz stimuli. A very stable effect however could be observed for the factor *component*: all ECD of SSF were situated further medial than their N1m counterparts ($F_{1,10} = 7.10$, $p < .05$). No effects for the factor *paradigm* or interactions were observed (all $Fs < 1$).

As can be taken from Table 2 and Figure 3 (lower panel), all sources of the SSF components were more superior located than those of the N1m ($F_{1,10} = 7.28$, $p < .05$). However there was also a dependence of the difference on the factor *paradigm*, which is indicated by a significant *component* x *paradigm* interaction ($F_{1,10} = 7.47$, $p < .05$). A separate ANOVA for each component showed that the SSF-source of the combined paradigm were located superior to the one related to the classical paradigm ($F_{1,10} = 10.11$, $p < .01$).

Overall, the results of ECD analysis suggest distinct representations for the components and the paradigms with a main tonotopic gradient in the anterior-posterior direction. The SSF is generated more medial (≈ 5 mm) and more superioral (≈ 3 mm, depending on the paradigm) than the N1m. Sources from the combined paradigm were consistently located more anterior (≈ 1.6 mm) than those elicited by the classic paradigm. For the purpose of visualization the mean sources collapsed over frequencies was plotted onto a standard brain (average of 24 Talairach transformed brains used in BESA 2000 [MEGIS Software GmbH, Munich, Germany]), which is shown in Figure 4.

Dipole Orientation

The angle of the right-hemispheric N1m-dipole decreased (rotates clockwise) with increasing carrier frequency (see Figure 5). This is also true for the SSF-dipole orientation derived from the combined condition but contrasted by SSF-classic which produced a counter-clockwise rotation of dipole orientation with increasing carrier frequency. For the results of the combined paradigm, an ANOVA resulted in a

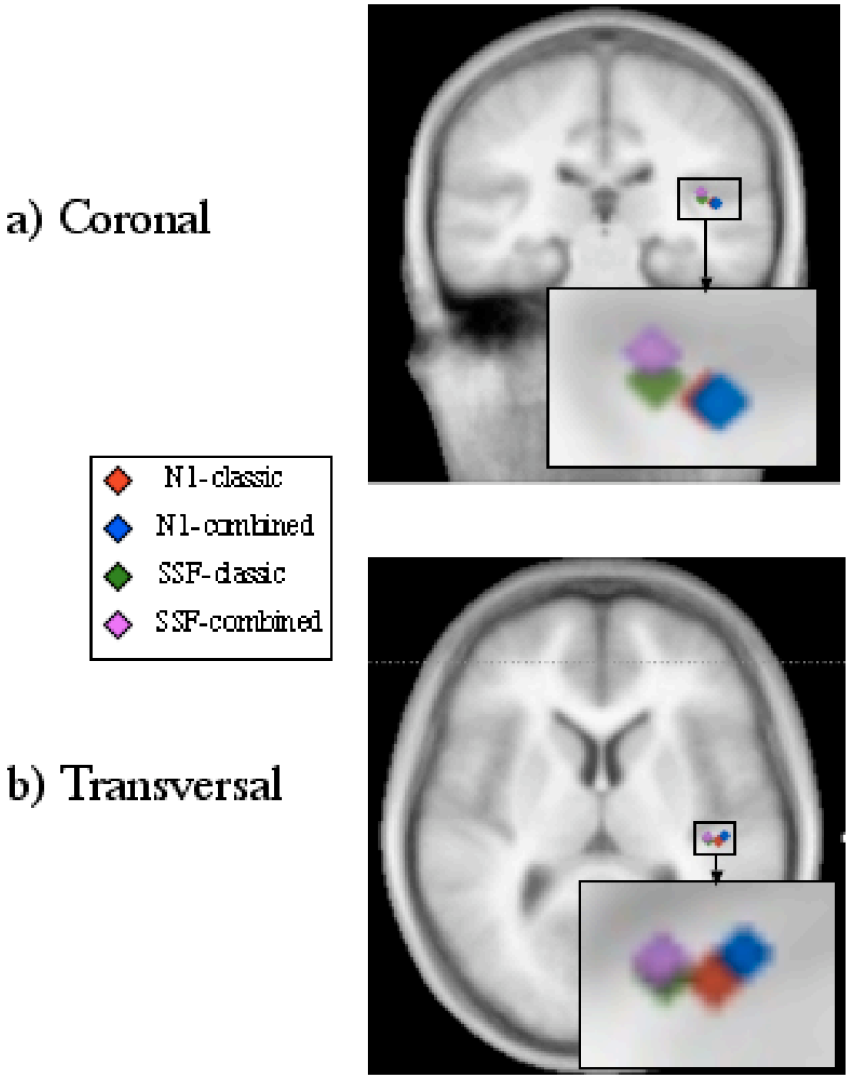

Figure 4: Overlay of each *component x paradigm* condition collapsed over all carrier frequencies.

significant effect of frequency ($F_{3,27} = 3.11$, $p < .05$). Post-hoc analyses showed that this effect was largely due to a significantly less negative angle for 1000 Hz as compared to 6000 Hz (7.33°; critical difference: 7.32°). The comparison between 1000 Hz and 3302 Hz approached significance (6.80°).

Discussion

Compared to other MEG-studies the direction of tonotopy we found is rather unusual, yet not surprising. It is in agreement with the study by Elberling et al. (1982), animal studies (see Kaas et al., 1999) and functional imaging studies (Lauter et al. 1985; Wessinger et al., 2001). From neuroanatomy it is also known that Heschl's gyrus does not only extend medially, but also in a posterior direction (Talavage et al, 2000). The

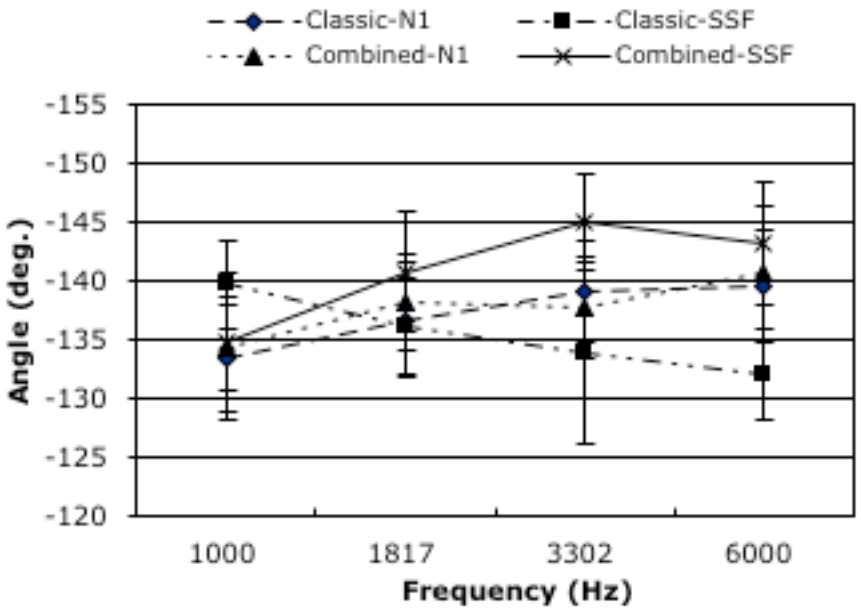

Figure 5: Dipole orientation in the saggital plane. Increasing negative values correspond to a clockwise movement when viewed from the right side of the brain.

systematic relationship between the carrier frequency and the dipole orientation increases our confidence that tonotopy was assessed. The inability to find a medial-lateral tonotopic gradient, does not indicate that it was generally absent. Several factors might have contributed to this situation. One of them concerns the apparatus employed: Using a magnetometer we can not exclude the possibility that sensors contralateral to the stimulation measure some ipsilateral activity. This would lead to enhanced variability in the medial-lateral direction. Another point is that assuming a single active neuronal generator at each time point for the sake of localization most surely oversimplifies the actual source configuration. In fact there has been some evidence, at least two sources are involved in the generation of both – the N1m (Rogers et al., 1990) and SSF (Gutschalk et al., 1999) – components. This should be kept in mind when interpreting the present results.

The more medial localization of the SSF is in accordance with previous studies (Godey et al., 2001) and gives support to the assumption that the SSF and N1m sources stem from different auditory fields. In addition to this finding, several other aspects of the results replicated across paradigms: Concerning peak latency and amplitude of the N1m, SSF phase, and SSF amplitude, it is interesting that the combined and the classic approach showed the same pattern of results. Specifically,

we replicated the previously reported (Roß et al., 2000) negative relationship between SSF carrier frequency and amplitude / phase.

Next to these parallels however, several systematic differences between the paradigms indicate that the tonotopic maps derived via the two approaches are not entirely the same. These differences were observable for amplitude and latency characteristics of the respective waveforms, with N1m peaking earlier and with higher amplitude in the classic condition. Furthermore the aforementioned amplitude and phase relationships with carrier frequency for the SSF were more pronounced for the classic condition as indicated by higher contrast F-values and effect sizes. It has to be mentioned of course that more than twice as many epochs were obtained in the classic than for the combined paradigm. So this difference might be mainly due to a higher signal-to-noise ratio in the classic condition. Certainly, most informative concerning the assumption of diverse tonotopic fields are the results of the ECD analysis. For the N1m, source strengths were largest when elicited by the classic (i.e. pure tone) paradigm. Localization of the ECDs were markedly different for the two paradigms especially in the anterior-posterior direction with sources of the combined paradigm lying consistently further anterior. Based on a sample of our subjects, we can say that localization accuracy for the N1m is excellent in the majority of cases with confidence intervals around 30 mm^3. This magnitude conforms well with results from a simulation study reported by Gallen et al. (1992). Due to the averaging procedure for the SSF (without baseline) an estimate of confidence intervals are not possible for this component. Taken together with the N1m-SSF comparison it is possible to speculate that each paradigm x component combination provokes activity from different auditory fields. At least three fields of the auditory cortex – AI and the rostrotemporal field of the core and the middle-lateral field of the belt area – are known to exhibit a low-to-high anterior-posterior gradient as observed in this study Kaas et al., 1999; Hackett et al., 2001). Yet it is unclear to what extent these results mainly gained from investigating macaque brains are applicable to humans. Parametric studies using fMRI and MEG on the same set of subjects, appear to be promising to investigate the question left open by our study, that the different conditions elicited activity from different auditory fields, as a) the amount of auditory fields in humans and also b) the stimulus features necessary to evoke activity from these fields can be investigated. However also more trivial alternatives have to be

taken into account. In case of the steady state it is e.g. conceivable that sources of P2m- (Lütkenhöner and Steinsträter, 1998; Tarkka et al., 1995) and sustained field (SF; Mäkeli and Hari, 1987) contributed more to the further anterior lying localization for the combined condition, due to its shorter duration as compared to the classic condition. To conclude, the present study showed that two tonotopic maps can be obtained simultaneously by combining features of standard N1m (evoked field) and SSF paradigms. Very remarkable are the identical patterns of results concerning amplitude and latency / phase characteristics of the components. However, we found significant differences between the combined and the standard N1m and SSF paradigm in contrast to the result of Engelien et al. (2000). A possible explanation concerns the choice of frequencies. In contrast to Engelien et al. who used a low (250 Hz) carrier frequency, we focused on high carrier frequencies (> 1000 Hz). Following studies should address this question by including additionally lower carrier frequencies. An additional effect would be to have a wider range of octaves than in the present study which might have also contributed to the missing medial-lateral tonotopic gradient. In general, effects were more pronounced when gained via the classic paradigms. As in the case of the SSF this however might simply be a result of a smaller signal-to-noise ratio of the bi-mapping paradigm. Even if more trials than presently used are recommended, we consider the design to be of some impact for clinical routine. For instance, it seems ideally suited to study plastic alterations that have been observed in blind individuals (Elbert et al., 2002) or subjects with a hearing loss (Mäkelä et al., 2000).

References

Ehret G. The auditory cortex. J Comp Physiol 1997; 181: 547-557.

Elberling C, Bak C, Kofoed B, Lebech J, Saermark K. Auditory magnetic fields: source location and ‚tonotopical organization' in the right hemisphere of the human brain. Scand Audiol 1982; 11: 61-65.

Elbert T. Sterr A, Rockstroh B, Pantev C, Müller, M, Taub E. Expansion of the tonotopic area in the cortex of the blind. J Neuroscience 200*2;* 22: 9941-9944.

Engelien A, Schulz M, Ross B, Arolt V, Pantev C. A combined functional in vivo measure for primary and secondary auditory cortices. Hear Res 2000; 148: 153-160.

Galambos R, Makeig S, Talmachoff PJ. A 40-Hz auditory potential recorded from the human scalp. Proc Natl Acad Sci 1981; 78: 2643-2647.

Gallen C, Pantev C, Hampson S, Buchanan DS, Sobel D. Reliability and validity of auditory neuromagnetic source localization using a large array biomagnetometer. In Manfred Hoke, Sergio Nicola Erné, Yoshio Okada and Gian Luca Romani (eds.): Biomagnetism: Clinical Aspects (pp. 171-175). Amsterdam: Elsevier, 1992.

Godey B, Schwartz D, de Graaf JB, Chauvel P, Liégeois-Chauvel C. Neuromagnetic source localization of auditory evoked fields and intracerebral evoked potentials: a comparison of data in the same patients. Clin Neurophysiol 2001; 112: 1850-1859.

Gutschalk A, Mase R, Roth R, Ille N, Rupp A, Hahnel S, Picton TW, Scherg M. Deconvolution of 40 Hz steady-state fields reveals two overlapping source activities of the human auditory cortex. Clin Neurophysiol 1999; 110: 856-868.

Hackett TA, Preuss TM, Kaas JH. Architectonic identification of the core region in auditory cortex of macaques, chimpanzees, and humans. J Comp Neurol 2001; 441: 197-222.

Hämäläinen M, Ilmoniemi RJ. Interpreting measured magnetic fields of the brain: estimates of current distributions. Technical Report TKK-F-A559, 1984.

Hari R, Hämäläinen M, Joutsiniemi SL. Neuromagnetic steady-state responses to auditory stimuli. J Acoust Soc Am 1989; 86: 1033-1039.

Hauk O, Keil A, Elbert T, Müller M. Comparison of data transformation procedures to enhance topographical accuracy in time-series analysis of the human EEG. J Neurosci Methods 2002; 113: 111-122.

Kaas JH, Hackett TA, Tramo MJ. Auditory processing in primate cerebral cortex. Curr Opin Neurobiol 1999; 9: 164-170.

Lauter JL, Herscovitch P, Formby C, Raichle M. Tonotopic organization in human auditory cortex revealed by positron emission tomography. Hear Res 1985; 20: 199-205.

Lütkenhöner B, Steinsträter O. High-precision neuromagnetic study of the functional organization of the human auditory cortex. Audiol Neurootol 1998; 3: 191-213.

Mäkelä JP, Hari R. Evidence for cortical origin of the 40 Hz auditory evoked response in man. Electroenceph Clin Neurophysiol 1987, 66: 539-546.

Mäkelä JP, Elbert T, Kakigi R, Lopez L, Nagamine T, Nakasato N, Sannita W. MEG: Clinical applications. In: Aine C, Okada Y, Stroink G, Swithenby S, Wood C, editors, Advances in Biomagnetism Research: Biomag96. New York: Springer, 2000: 980-990.

Merzenich MM, Knight PL, Roth GL. Representation of cochlea within primary auditory cortex in the cat. J Neurophysiol 1975, 38: 231-249.

Pantev C, Elbert T, Makeig S, Hampson S, Eulitz C, Hoke M. Relationship of transient and steady-state evoked fields. Electroenceph Clin Neurophysiol 1993, 88: 389-396.

Pantev C, Roberts LE, Elbert T, Roß B, Wienbruch C. Tonotopic organization of the sources of human auditory steady-state responses. Hear Res 1996, 101: 62-74.

Pastor MA, Artieda J, Arbizu J, Marti-Climent JM, Penuelas I, Masdeu JC. Activation of human cerebral and cerebellar cortex by auditory stimulation at 40 Hz. J Neurosci 2002, 22: 10501-10506.

Rogers RL, Papanicolaou AC, Baumann SB, Saydjari C, Eisenberg HM. Neuromagnetic evidence of a dynamic excitation pattern generating the N100 auditory response. Electroenceph Clin Neurophysiol 1990; 77: 237-240.

Romani GL, Williamson SJ, Kaufman L. Tonotopic organization of the human auditory cortex. Science 1982, 216: 1339-1340.

Romani GL, Williamson SJ, Kaufman L, Brenner D. Characterization of the human auditory cortex by the neuromagnetic method. Exp Brain Res 1982, 47: 381-393.

Romani GL. Tonotopic organization of the human auditory cortex revealed by steady state neuromagnetic measurements. Acta Otolaryngol Suppl 1986, 432: 33-34.

Rosenthal R, Rosnow RL. Contrast analysis: focused comparisons in the analysis of variance. Cambridge: Cambridge University Press, 1985.

Roß B, Borgmann C, Draganova R, Roberts LE, Pantev C. A high-precision magnetencephalographic study of human auditory steady-state responses to amplitude modulated tones. J Acoust Soc Am 2000; 108: 679-691.

Rouiller EM. Functional organization of the auditory pathways. In Günter Ehret and Raymond Eomand (eds.): The central auditoy system (pp. 3-96). New York: Oxford University Press, 1997.

Talavage TM, Ledden PJ, Benson RR, Rosen BR, Melcher JR. Frequency-dependent responses exhibited by multiple regions in human auditory cortex. Hear Res 2000, 150: 225-244.

Tarkka IM, Stokic DS, Basile LF, Papanicolaou AC. Electric source localization of the auditory P300 agrees with magnetic source localization. Electroencephalogr Clin Neurophysiol 1995; 96: 538-545.

Tiihonen J, Hari R, Kaukoranta E., Kajola M. Interaural interaction in the human auditory cortex. Audiology 1989; 28: 37-48.

Tiitinen H, Alho K, Houtilainen, Ilmoniemi RJ, Simola J, Näätänen R. Tonotopic auditory cortex and the magnetencephalographic (MEG) equivalent of the mismatch negativity. Psychophysiology 1993; 30: 537-540.

Verkindt C, Bertrand O, Perrin F, Echallier JF, Pernier J. Tonotopic organization of the human auditory cortex: N100 topography and multiple dipole model analysis. Electroencephalogr Clin Neurophysiol 1995; 96: 143-156.

Victor DJ, Mast J. A new statistic for the steady-state evoked potential. Electroencephalogr Clin Neurophysiol 1991; 78: 378-388.

Wallace MN, Johnston PW, Palmer AR. Histochemical identification of cortical areas in the auditory region of the human brain. Exp Brain Res 2002; 143: 499-508.

Wessinger CM, VanMeter J, Tian B, Van Lare J, Pekar J, Rauschecker JP. Hierarchical organization of the human auditory cortex revealed by functional magnetic resonance imaging. J Cogn Neurosci 2001; 13:1-7.

Appendix B

Manuscript of:

Tonotopic organization of the human auditory cortex probed with frequency-modulated tones

Weisz, N., Wienbruch, C., Hoffmeister, S. & Elbert, T.

Submitted to *Hearing Research*
Accepted for publication: 9.1.2004
Current status: in press

Abstract

Using neuromagnetic source imaging, we investigated tonotopic representation and direction sensitivity in the auditory cortex of humans (N = 15). For this purpose, source analysis was undertaken at every single sampling point during the presentation of a frequency-modulated tone (FM) sweeping slowly downward or upward across periods of 3s duration. Stimuli were selected to target response properties of the central part of the primary auditory cortical field, which has been shown to exhibit sensitivity to distinct FM-sound features as compared to the ventral and dorsal part. Linear mixed-effects model statistics confirm tonotopic gradients in medial-lateral and anterior-posterior directions. The high resolution provided by this method revealed that the relationship between frequency and spatial location of the responding neural tissue is nonlinear. The idea that neurons specifically sensitive to the employed sound characteristics (slow, downward modulation) were activated is supported by the fact that the upward sweep of identical duration produced a different pattern of functional organisation.

Keywords: Tonotopy, MEG, high-resolution, LME

Introduction

A fundamental organizational principle of the central auditory system is the frequency specific (tonotopic) representation of neuronal populations (de Ribaupierre, 1997; Horikawa et al., 2001; Schreiner et al., 2000), preserving the arrangement of hearing receptors in the inner ear (high-to-low frequency representation from base to apex). This means that neurons processing information from neighbouring hair cells are also neighbours on a tonotopic map. On a cortical level, several fields exist that process auditory information, reflecting the hierarchical and parallel processing architecture of the auditory cortex. The exact number of fields is unknown for humans (Ehret, 1997; Talavage et al., 2000; Wallace et al., 2002). However, one can expect the situation to be similar to non-human primates, for which Kaas et al. (1999) proposed a rough subdivision of the auditory cortex into three areas: the core (primary auditory cortex; incorporating the primary auditory cortical field, AI), the belt (secondary auditory cortex; including the secondary auditory cortical field, AII), and the parabelt (Kaas et al., 1999). In general, the degree of tonotopy decreases and the degree of multimodal integration increases in the order mentioned above.

Tonotopy is most frequently investigated by measuring neuronal responses to various stimuli consisting of a single carrier frequency, typically a pure tone. In humans, magnetencephalography (MEG; e.g., Elbert et al., 2002; Pantev C., 2003) has been a frequently employed technique for noninvasively investigating the functional organisation and reorganisation of the auditory cortex (for functional neuroimaging approaches see e.g., Lauter et al., 1985; Lockwood et al., 1999; Wessinger et al., 2001).

The conventional MEG-approach attempts to model the magnetic signals measured from the surface of the head by modelling the underlying source with a single equivalent current dipole (ECD) per hemisphere (see. e.g. Elbert, 1998; Hämäläinen et al., 1993). A frequency-dependent spatial shift in the location of the ECD is considered as an indicator for tonotopic organization. This strategy has been frequently employed for various neuromagnetic components, e.g. the N1 (Godey et al., 2001; Lütkenhöner et al., 2003; Pantev et al., 1988; Pantev et al., 1995), the Pa (Pantev et al., 1995) the steady state field (SSF) (Engelien et al., 2000; Pantev et al., 1996; Ross et al., 2000) and the sustained field (Pantev et al., 1994).

However, this approach has important shortcomings:

1) In order to gain an acceptable signal-to-noise ratio the same stimulus must be presented at a slow rate (less than 1/sec) and with many repetitions, thus imposing constraints on the number of frequencies that can be investigated (usually only three or four).
2) The power of the N100 peak is in the frequency range below 12-15 Hz, i.e., the signal is contaminated by coherent biological noise that significantly distorts source modelling.
3) Natural auditory stimuli to be processed by the auditory cortex are generally complex and time-variant. Since the study of complex tones is a difficult endeavour the use of amplitude and frequency modulated tones poses an attractive alternative (Tian et al., 1994): they are not as artificial as pure tones, yet their physical dimensions are more easily controlled than natural sounds.

The third point is reflected in the fact that FM tones provoke stronger neuronal responses than responses to the respective characteristic frequencies (CF) (deCharms et al., 1998; Nelken et al., 1994). DeCharms et al. (1998) demonstrated, for example, that only 5 % of investigated neurons in AI are exclusively responsive to a single frequency region. The great majority of neurons show sensitivity to additional features. This is also reflected in the organization of the the tonotopic map of AI in isofrequency stripes (dorsal, central and ventral) at similar CF as shown in animal data (Ehret, 1997; Schreiner et al., 2000). Each stripe combines a different mixture of response characteristics to certain features of acoustic stimuli (binaural interaction, monotonicity of rate-intensity function, dynamic range etc.).

For frequency-modulated tones (FM-sweeps), i.e. tones with a constantly changing instantaneous frequency, these features are mainly direction, speed and range. Concerning direction and speed the central isofrequency stripe shows distinct response properties with a majority of neurons (about 2/3) preferring downward sweeping tones (i.e. higher to lower frequencies) and slow modulation speeds (Heil et al., 1992b; Mendelson et al., 1985; Mendelson et al., 1993; Orduna et al., 2001; see Tian et al., 1994 for an investigation of neuronal responses to FM-sweeps in the anterior auditory field). The dorsal and ventral stripe show a directly opposite response pattern. Direction and speed preference has been attributed to a common mechanism (Gordon et al., 1998): an asymmetric distribution of inhibitory sidebands of an excitatory tuning curve around a CF. Heil and coworkers (1992c) were able to

demonstrate an orderly arrangement of FM direction selective neurons in A1 that parallels the characteristic frequency: regions sensitive to low tones preferred upward sweeps, whereas neurons with a high characteristic frequency seem to prefer downward sweeps. These results were recently corroborated by Zhang et al. (2003).
Of interest is also the observation that neurons in the central stripe have the narrowest frequency tuning curves and the lowest scatter of CF corresponding to the greatest frequency selectivity on a single- and multiunit level (Ehret, 1997; Heil et al., 1992a; Schreiner et al., 2000). This means that sweep ranges exceeding the excitatory bandwidth of the frequency tuning curve at a certain CF should lead to a corresponding spatial shift in the focal point of neuronal activity along the tonotopic gradient. The observation of neuronal activation being triggered when the sweep crosses the CF of the neuronal cluster has been shown in animal experiments (Nelken et al., 2000). More specifically, responses seem to be evoked by modulations towards the CF of neurons, i.e. before instantaneous frequency reached CF, and not by modulations away from the CF (Heil et al., 1992b). Employing a voltage sensitive dye, Horikawa et al. (1998) were able to show a spot-like response crossing the isofrequency bands in the primary auditory cortex, thus 'traveling' along the tonotopic axis. So far, a similar documentation of spatiotemporal activation induced by FM-stimuli has not been attempted in humans. The present study exploited the excellent temporal resolution capacity of MEG (Elbert, 1998; Hämäläinen et al., 1993) in order to investigate whether ECD sources alter their location in a systematic fashion during the processing of FM-sweeps. Data concerning neuronal coding of sweep rate and direction stem essentially from work on nonhuman primates (e.g., cats, bats etc.). In the process of designing our stimuli, efforts were undertaken that they matched response preferences of the central stripe of AI as described by Ehret (1997). Therefore, sweep direction was downwards from 8 to 0.5 kHz with an slow modulation frequency of 1/6 Hz (downward slope of sinusoidal FM tone; corresponding to an average frequency change of 2.5 kHz/s). This – for animal standards – unusually slow speed of modulation spanning across 3 seconds was mainly motivated by methodological reasons: a) It can not be expected that magnetic source imaging can adequately resolve shifts in activation along a tonotopic gradient, when changes become widely dispersed due to a fast spread of activation. b) A longer sweep duration allows a low-pass filter that eliminates spontaneous coherent noise

(alpha activity) and leads to a greater number of data points that enter the statistics (see below). The estimation of tonotopic gradients will be more robust against outlying values. A control condition kept everything constant except for sweep direction, which went upward from 0.5 to 8 kHz (upward slope of sinusoidal FM tone). This condition was expected to yield considerably worse localizations than the experimental condition due to the fact that sound features were not optimized for a presumed region equivalent to AI in animal studies.

Various criteria concerning localization and goodness of fit (GOF) were applied to the individual data (see method section for criteria) to exclude source localizations of low quality. From 15 original datasets only 2 had to be excluded for the downward sweep condition. For the upward sweep condition, however, data generally could *not* be modelled by a single source per hemisphere, indicating that source configurations for this case were complex (12 of the 15 subjects). Consequently, statistical analysis was only performed for the downward sweep data.

A linear mixed-effects model (LME) approach (Pinheiro et al., 2000; see also Goldstein, 2003) was used to specify fixed- (i.e., average population characteristics) and random-effects (i.e., subject variability). This statistic is frequently employed in studies with repeated measures: among the advantages of this approach are that it can a) treat continuous data, b) the amount of data may vary among subjects (in our case: a different number of acceptable dipole fits per person) and c) it returns coefficients for the terms in our models (fixed-effects). To test the coefficients of the fixed-effects parts, different measures were used (i.e., conditional t-test for linear regression models). Furthermore, models (i.e., different fixed effects described in the Methods section) were compared regarding which one is more appropriate in describing the data (Akaike Information Criterion [AIC] and Likelihood Ratio Test [LRT]). For a detailed account of the LME approach the reader is referred to Pinheiro et al. (2000).

Methods

Participants

Fifteen subjects (age: range =17-32 years, mean = 26, SD = 4.21; 9 males) with normal hearing and no history of an audiological or neurological disorder gave written informed consent to participate in the study. They received 15 € for participation. The study was approved by the Konstanz University Ethical Review Board.

Neuromagnetic recordings

Neuromagnetic data were recorded (sampling rate: 678.17 Hz; 0.1 – 100 Hz analogue filter) using a 148 channel whole-head magnetometer (4D Neuroimaging Inc., San Diego). Vertical and horizontal eye-movements (EOG) were measured from above and below the eye and from the outer canthi. Epochs contaminated by eye-movements or eye-blinks were not included in the averaged brain responses.

Procedure

Auditory stimuli were monaurally presented via a plastic tube attached to an earpiece in the left ear of the participant. Individual sensation levels (SL) were assessed for four pure tones (500 ms; frequencies: 500, 1000, 3750 and 8000 Hz). The experimental stimuli were presented at intensities approximately 50 dB above SL. During the neuromagnetic recording session, subjects laid in a supine position while watching a film of their choice that was projected onto the ceiling of the magnetically shielded room.

The frequency-modulated (FM) tones were generated with Matlab (version 5.2; The MathWorks, Inc.) according to the formula by (Hartmann, 1998):

$$x(t) = sin(\omega_c t + (\beta\, sin(\omega_m t + \phi))),$$

where x(t) denotes the amplitude x at time t, ω_c is the constant carrier frequency, ω_m the modulation frequency, ϕ instantaneous phase and β the modulation index.

Two blocks of FM-tones (3000 ms duration; 275 epochs; 4000 - 4200 ms random SOA) were presented to the participants in a counterbalanced order. The FM-tones in the blocks differed in their sweep-direction: In the upward-condition they changed from 0.5 – 8 kHz (upward ramp of frequency), in the downward-condition from 8 – 0.5 kHz respectively (downward ramp). Due to the sinusoidal modulation, the sweep was changing the frequency at slower rates during the beginning and during the end of the stimulus (approx. 2 kHz/s) but had a faster rate of change in the middle part (approx. 3.5 kHz/s).

Data-analysis

Epochs of 4000 ms length (100 ms baseline) were extracted from the raw data. Artefact free epochs, defined as EOG and MEG amplitudes below 100μV or 5 pT respectively, were averaged and filtered with a 0.7 Hz digital low-pass (Butterworth

characteristic of 5th order) in order to obtain the continuous response along the tonotopic gradient of an auditory field(s) sensitive to the stimulus features applied. For each subject a group of 25 – 38 magnetic sensors was determined over the right hemisphere that covered best the evoked magnetic fields from one hemisphere. Single equivalent current dipoles were fitted for every sampling point during tone presentation (i.e. 0 – 3000 ms).

Solutions were discarded from further analysis if their goodness of fit (GOF) was below .90, their localizations (BTI-coordinates) was more lateral than –7 cm or more medial than –1.5 cm, and more posterior and anterior than –4 cm or 4 cm respectively. Furthermore, a dataset was only considered as qualitatively sufficient when solutions meeting the described criteria could be found for the majority of sampling points (i.e., > 50 %). This left 13 participants for the downward-sweep condition and 3 for the upward sweep.

Because the downward-sweep FM was the only condition in which a sufficiently high number of results as well as stable results were acquired, inferential statistics were only applied to this condition. For this purpose we chose an inside-out approach: In a first exploratory step, orthogonal polynomials of degree 1 to 5 were fitted to the individual data. This was done to decide which terms should enter the fixed-effects part of a linear mixed-effects model (LME) (Pinheiro et al., 2000) being used for the group statistics. Starting with a single linear term, the gain in explained variance was observed when adding additional terms of the next higher polynomial. The five models tested were:

$$(1)\ y = I + ax,$$

$$(2)\ y = I + ax + bx^2,$$

$$(3)\ y = I + ax + bx^2 + cx^3,$$

$$(4)\ y = I + ax + bx^2 + cx^3 + dx^4,$$

$$(5)\ y = I + ax + bx^2 + cx^3 + dx^4 + ex^5,$$

where y is the response variable (i.e., the x-, y- and z-direction), x the covariate (here: latency) and a – e the coefficients.

Two criteria were introduced in order to select models for subsequent statistics and to keep assumptions concerning the parameters of the model to a minimum, i.e. to determine a cut-off beyond it makes little sense to add additional terms:

(1) A repeated measure ANOVA with the factor degrees of polynomials was calculated for the dependent variable R^2. In case of a significant effect, post-hoc analysis (Tukey-Kramer procedure) was performed to show which comparisons were statistically different, beginning with a first order polynomial moving upward in one-degree steps (i.e. comparison: degree 1 vs. 2, 2 vs. 3, etc.). Comparisons ceased when adding another term did not enhance R^2 in a significant manner.

(2) In addition, Cohen's (1998) effect size criteria served for judging whether R^2-enhancements were practically important. For this, each additional term had to raise overall goodness of fit by at least 0.09 (corresponding to a medium effect size).

This led to the selection of models 1 – 3. These were entered separately as fixed effects of a linear mixed effects model statistic (LME). Random effects were defined as subject variability for the parameters. Prior to calculation latency was centered at medium latency and scaled by factor 1000. Parameter estimation was performed using maximum-likelihood (ML). Significance of individual coefficients was tested using conditional t-tests for linear regression models. Another procedure tested whether or not a better fit of the data might be a trivial effect of our model being too general (incorporating too many terms). Models were therefore compared among another with a) the Akaike Information Criterion (AIC), which penalizes models with too many parameters (this leads to higher AIC values; i.e. models with smaller AIC are to be preferred), and b) the likelihood ratio test (LRT) which considers the likelihood of a more general (L2) and a more restricted model (L1). For $2log(L2/L1)$ under the assumption that the restricted model is more adequate, the distribution of the LRT-statistic is a χ^2-distribution with k_2-k_1 degrees of freedom (k being the number of parameters estimated in the respective models). LME analysis was done using the nlme-library (Pinheiro et al., 2000) of R (Ross et al., 1996) (version 1.6.2) for Mac OS X.

Results

The mean (+/- SE) goodness of fit for the five regression models is depicted in Figure 1, which shows that a large amount of variance is explained by a single linear term (0.32 – 0.42; according to Cohen's (Cohen, 1988) classification a R^2 larger than 0.25

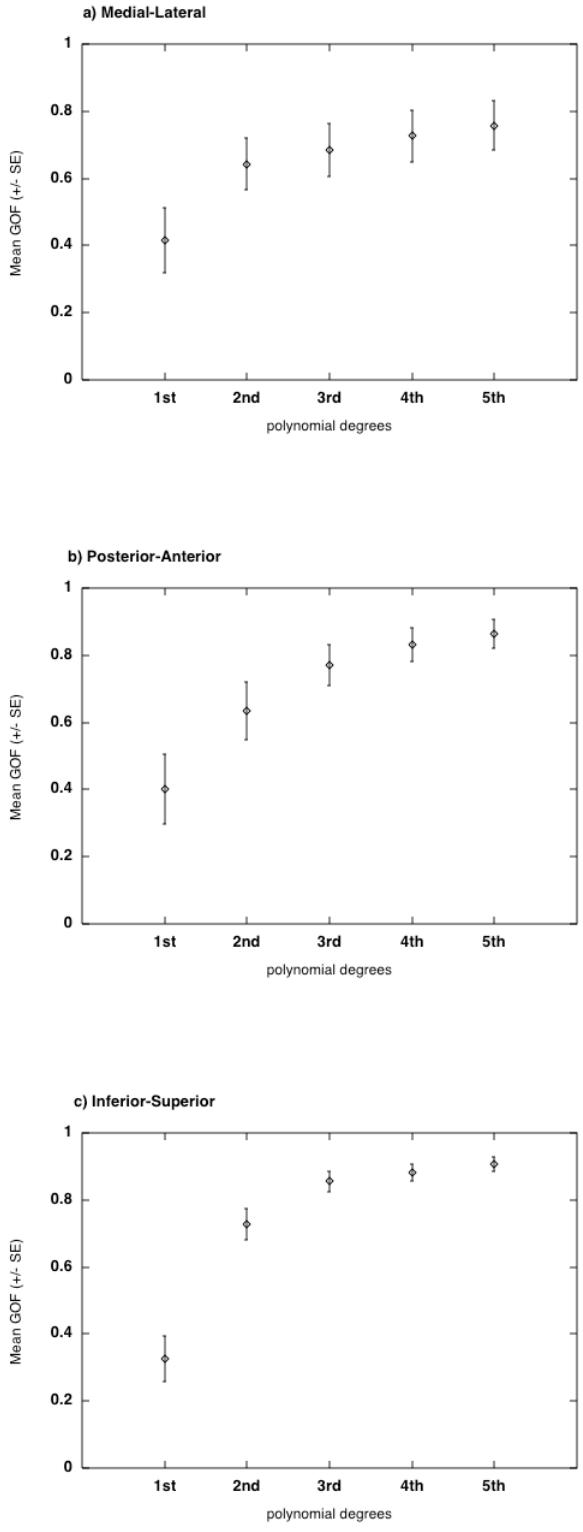

Figure 1: *Goodness of fit as a function of the number of polynomial parameters*. Plots show mean (+/- standard error) goodness of fit for increasingly complex polynomial fits (see models 1 – 5 in methods section) for the three spatial directions (medial-lateral, **a)**; posterior-anterior, **b)**; inferior-superior, **c)**).

represents a substantial effect size). Repeated measure ANOVAs indicate a significant effect for the factor polynomial degrees ($F_{4,48}$ for. medial-lateral: 14.53, for posterior-anterior: 19.31 and inferior-superior: 75.80; all $p < .001$). Post-hoc analysis showed statistically significant and practically important increases in R^2 in several cases:

- *Medial-lateral*: The addition of a quadratic term to a model with one linear term leads to a R^2-gain of 0.23 (critical difference: 0.14). A further cubic term does not contribute any significant extra information (R^2-gain: 0.04).
- *Posterior-anterior*: Same pattern as for medial-lateral with a large R^2-gain when adding a quadratic term (0.23; critical difference: 0.17) and a statistically not significant for a further cubic term (0.13).
- *Inferior-superior*: Enormous R^2-gain when adding a quadratic term (0.40; critical difference: 0.11). A statistically significant increase was also observed for an additional cubic term (0.13).

Based on these results two fixed-effects models were specified for the LME statistic for the medial-lateral and posterior anterior direction:

(1) Model 1: $y = I + ax$,

(2) Model 2: $y = I + ax + bx^2$,

where y is the response variable (x-, y- and z-axis), I the intercept, x the covariate (latency) and a and b the coefficients.

For the inferior-superior direction a third model was tested next to the other two described above:

(3) Model 3: $y = I + ax + bx^2 + cx^3$.

The observed data for each individual (P1-P13) are plotted as red dots for each coordinate in Figure 2. Due to the large amount of data only every 100th point is shown for the sake of clarity (the following LME statistic however include all data points). Additionally, data as predicted by the three models are depicted for each individual (Model 1: blue dots; Model 2: green dots; Model 3: purple dots). Two striking features can be noted from visual inspection of the data: First of all, there is a very high inter-subject variability. Secondly, the application of a linear fixed-effects model (i.e., Model 1; blue dots in Figure 2) does not seem appropriate in several cases.

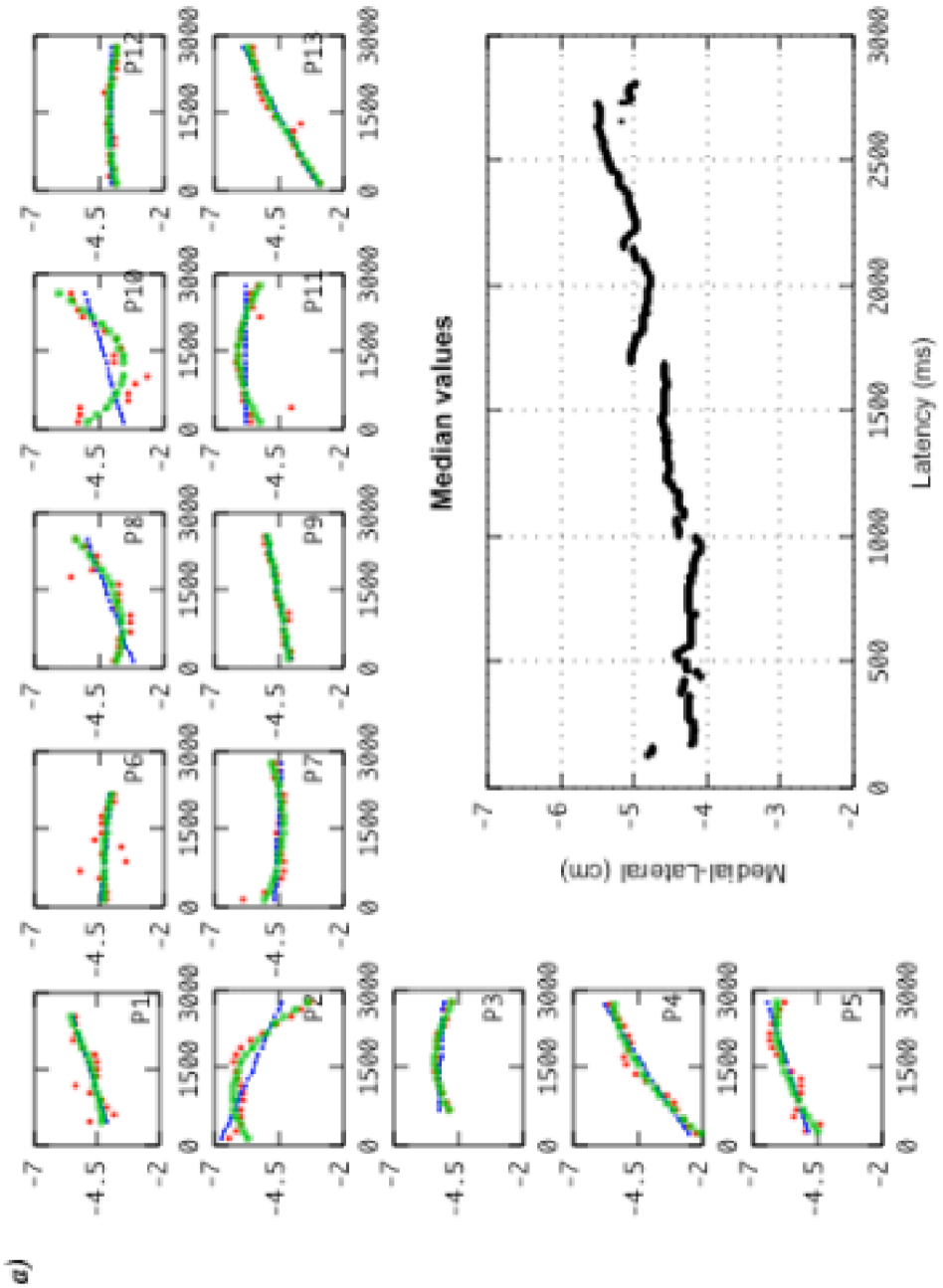

Figure 2: *Comparison of observed data and predicted values*. Large box depicts the median value at each sampling point included in the statistic. Small boxes include the observed data (**red dots**) and the values predicted by the different models (model 1: **blue dots**; model 2: **green dots**; model 3: **purple dots**) each individual (**P1 - P13**) by the LME statistic. Visualization was done for each direction separately ((medial-lateral, **a)**; posterior-anterior, **b)**; inferior-superior, **c)**). For better visibility only every 100th point is shown.

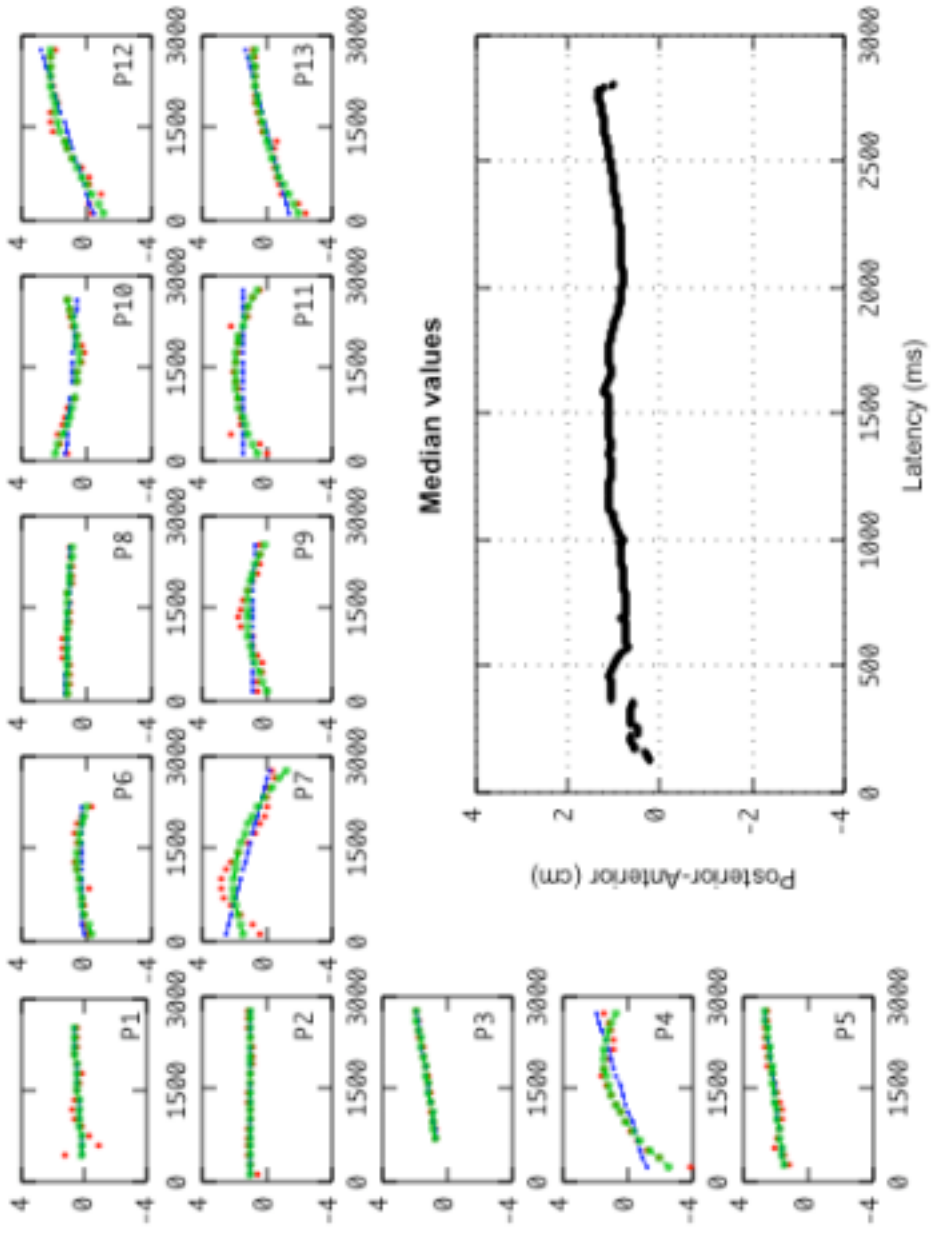

Figure 2 cont.

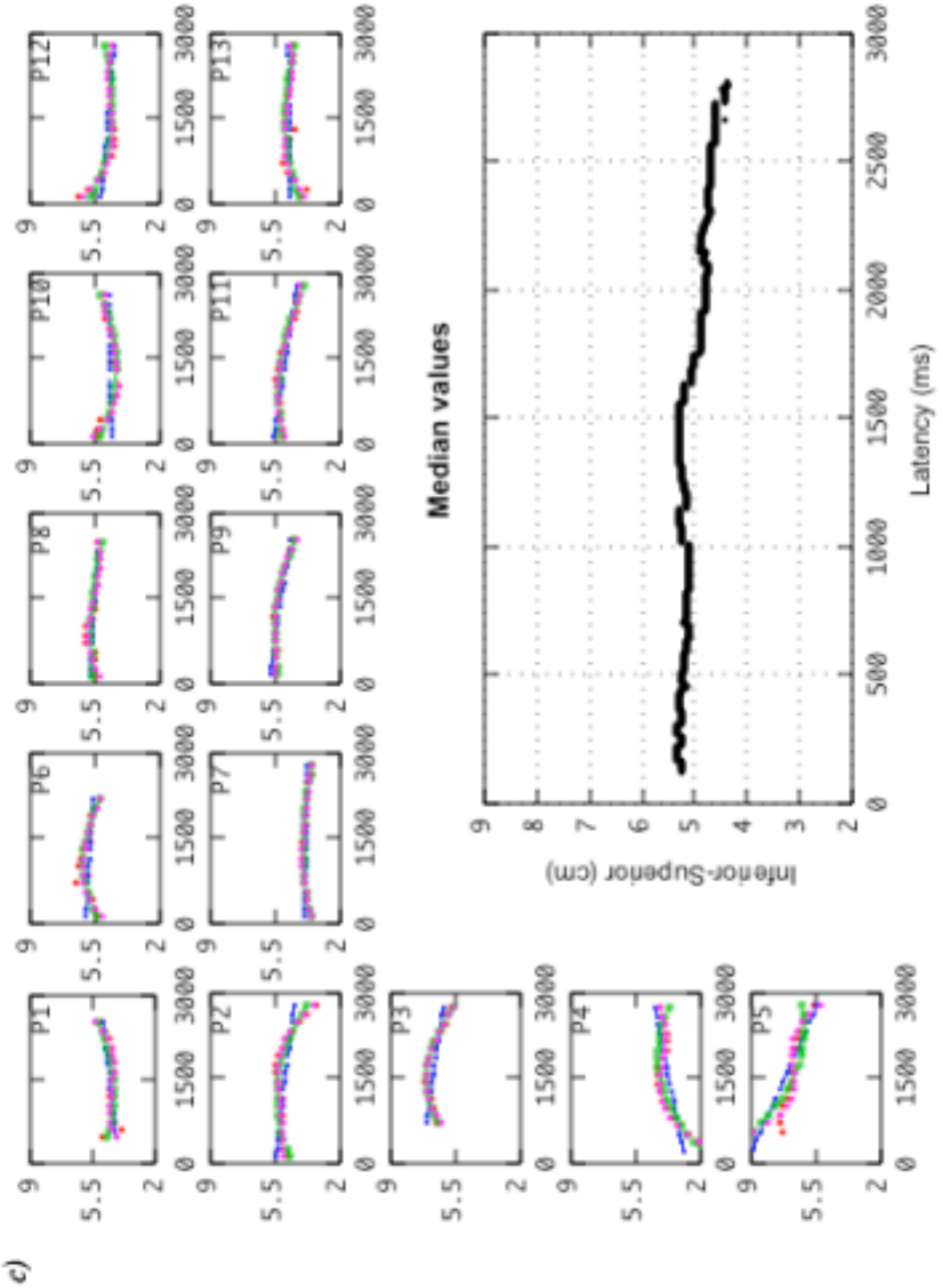

Figure 2 cont.

The latter impression is confirmed by the LME statistic (Table 1). Both statistical criteria, the AIC and LRT, indicate the superiority of models in which nonlinear (quadratic and cubic) terms are added (i.e., lower AIC- and higher LRT-values). Concerning the individual fixed-effects terms in the medial-lateral case, the linear part remains statistically significant regardless of the model tested (-0.32 and -0.36; $p < .05$). The negative sign of the coefficient means that overall source localization progressed laterally (towards the right) with time (model 2: from –4.10 cm to –5.17). For the posterior-anterior direction, there was no significant linear term, but a highly significant quadratic one (-0.37; $p < .01$). The negative sign of the quadratic fixed-effect part indicates a spatial shift of sources further anterior up to the middle of the time-window (model 2: from –0.05 cm to 1.14 cm) and a shift back towards posterior localizations from the middle until the end model 2: 0.65 cm). For the inferior-superior axis only the linear fixed-effect of model 3 approaches significance. In this case, the negative sign implies a further inferior source localization as a function of time. More specifically, as the nonlinear terms must be taken into account, the representations first shift superiorally up to about 980 ms (model 3: from 5.03 cm to 5.27 cm) and then towards inferior (model 3: from 5.27 cm to 4.64 cm). To gain an impression of this 'population'-tonotopy, we plotted source locations resulting from the LME-statistics (i.e., the coordinates derived from the fixed effect) on a standard brain (Figure 3).

Discussion

Concerning the tonotopic gradients, the models in this investigation are in accord with previous studies which have reported in most cases a medial to lateral and sometimes also a posterior to anterior shift of neural activity as the frequency decreases (Ehret, 1997; Elberling C, 1982; Engelien et al., 2000; Kaas et al., 1999; Pantev et al., 1989; Pantev et al., 1994; Pantev et al., 1996; Pantev et al., 1988; Pantev et al., 1995; Romani et al., 1982). Generally, such changes are observed for a subset of the sample only. The present study reveals the reasons: as the present analysis does not just "map" three or four points but many for each subject the individual variability becomes obvious as does a more complex, i.e., non-linear functional dependency between location and frequency. In addition, more inferior locations for low frequencies were indicated by the high resolution of the relationship. The considerable

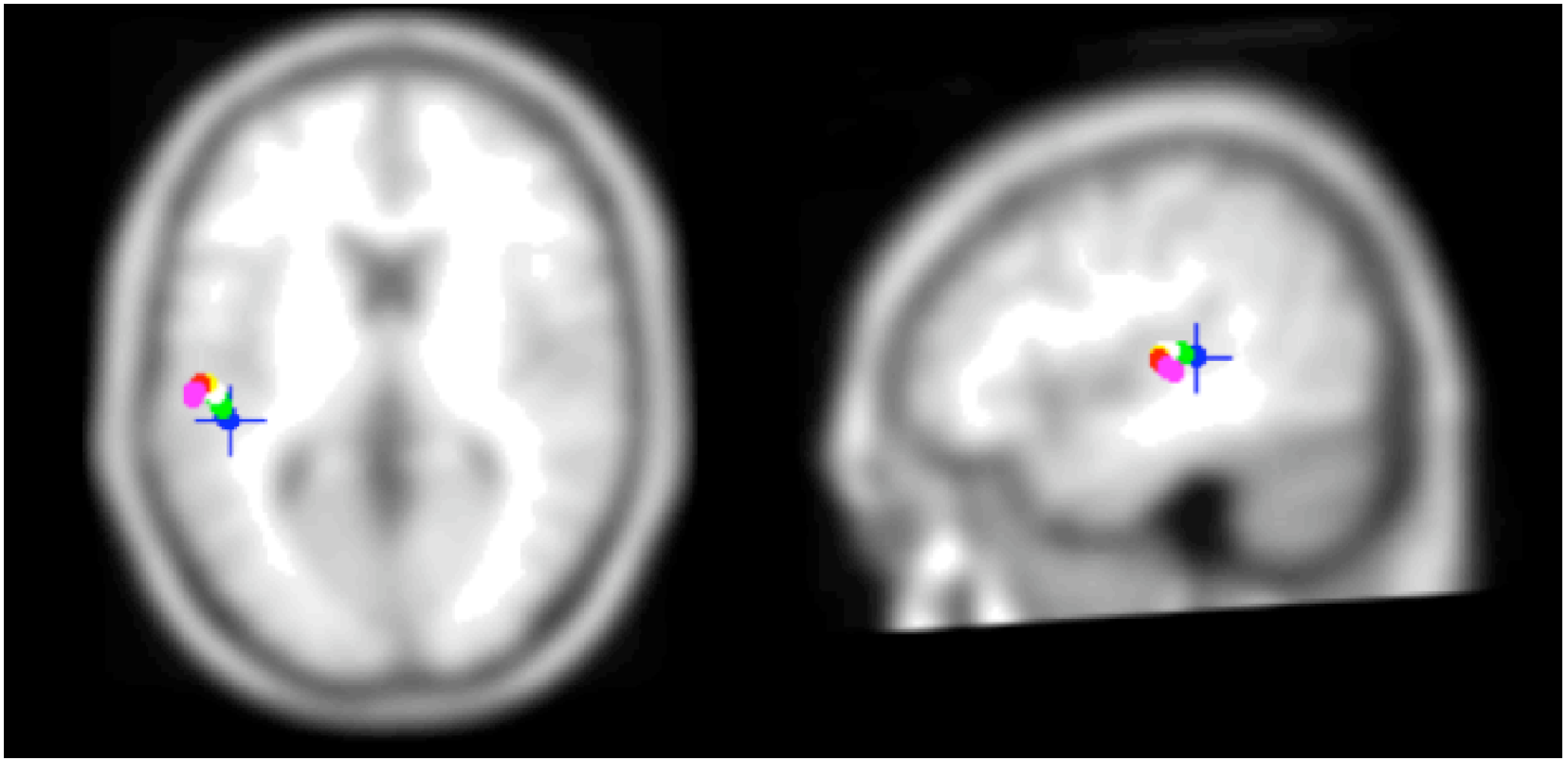

Figure 3: *MRI-Overlay of the fixed*. 1822 dipoles are plotted in total. 'Cold' colors (**blue**) indicate positions at early latencies (beginning at 124 ms post stimulus onset; corresponding to high frequencies). 'Warm' colors (**pink**) show positions at late latencies (ending 2809 ms post stimulus onset; corresponding to low frequencies). The cross indicates the location of the response to the stimulus onset (i.e., fit at 124 ms). Locations were obtained by using the coefficients of the most succesful models (fixed effect) obtained by the LME statistic (see. Table 1), i.e., this figure does not represent sources for a given individual but results from the group statistics.

inter-individual variability has been noted in animal studies (Ehret, 1997) and seems not surprising given the plasticity in representational cortex (Pantev et al., 2003). Ehret (1997) therefore argues that average statistics are inadequate and this indeed may pose a serious problem for several previous attempts to study tonotopy in humans. More recently, this question has also been recently raised by Lütkenhöner et al. (2003) from the perspective of neuroelectric and neuromagnetic studies of tonotopy. We demonstrated for our results how the LME statistic addresses this problem by taking random-effects into account.

The question remains as to which parts and mechanisms of the auditory cortex contributed to this finding. A trivial explanation, like the one suggesting that an overlap from N1 and sustained field contributed to the observed results, seems unlikely given that the time segment was three seconds. Moreover, comparable findings were not obtained for the upwards sweep. Generally, the specificity of the results for the downward sweep condition underlines the importance of the stimulus features employed. It seems possible that the downward sweep has been optimized for a certain area of AI, which exhibits this kind of direction-preference with slow modulation rates (analogue to the central stripe in cat AI; Ehret, 1997). That the data of the upward sweep could not be modelled with a single ECD per hemisphere is an indicator that at least two sources per hemisphere are involved, suggesting that different source configurations were active for the upward and for the downward sweep. This question however can not be solved with the currently available non-invasive methods. The limitations of the ECD analysis employed in the present study will be discussed below.

Furthermore, direction-sensitivity and asymmetric inhibitory side-bands of tuning curves seem to be related (Gordon and O'Neill, 1998; Nelken et al., 2000; Shamma et al., 1993). Opposite to FM-upwards-sensitive neurons, downwards-sensitive ones have stronger inhibitory side-bands on the low-frequency side of the tuning-curve. This means that a downward oriented sweep leads to a 'proper' sequence of excitation and inhibition when probing downward-sensitive neurons. Upward oriented sweeps would first lead to an inhibition of downward-sensitive neurons. These arguments (sweep direction / rate preference, asymmetry of inhibitory side-bands and their relation to direction sensitivity) make a case that the downward-oriented sweep lead

to a focal activation of the central part of AI that shifted along the tonotopic axis. Other explanations might arise, however, as we begin to understand more about the cortical processing of FM-sweeps, which until recently has been confined mainly to AI.

There are several factors that pose limitations to the interpretation of our results:

1) While designing the study, we relied on animal, mostly non-primate (cat) studies as reviewed by Ehret (1997). There are, however, strong species differences (for review see Eggermont, 2001), so in the case of humans it can not be excluded that subdivisions of AI and their response properties might not exactly match the ones assumed in the introduction. Nevertheless, the finding of acceptable ECD fits for the downward sweep for a wide time range point to a relatively focal activation within the auditory cortex. Future, probably invasive studies, will have to resolve the question whether there exist isofrequency stripes within AI with a strong preference for downward sweeps.
2) It has to be assumed that the processing of an FM tone will lead to activation in several auditory fields, even though it is unlikely that the stimuli employed lead to an equal and thus global activation in all fields (Eggermont, 2001). Thus source configurations can be too complex to be modelled by ECDs and may be to transient in time to be tracked by hemodynamic imaging methods. Only, if a certain cortical region dominates the pattern of neuromagnetic activation, a tontotopic gradient appears. This seems to be the case for the downward sweep.
3) It has to be kept in mind that the velocity of change in this study was extremely slow, complicating comparisons with results from animal studies. In cases where such slow-going FM rates have been investigated (cat studies), responsive neurons have been mainly found in the posterior auditory field (Heil et al., 1998; Tian et al., 1998) and even then peak responses were rather high compared to the rates used in the present investigation (< 100 and 200 kHz/s respectively).
4) Furthermore, it is difficult to ascribe unvarying response features to certain FM sweep characteristics (e.g. rate, range, direction) of auditory cortical neurons as they are also considerably dependent on other parameters. Heil et al. (1998) could show for neurons in the posterior auditory field of cats that

e.g., the rate of change of frequency (RCF) varied with sound pressure level (SPL) of the stimulus. Furthermore, the directional sensitivity changed with RCF and SPL. Also, the instantaneous frequency leading to a response of the neuron depended on SPL and FM direction. leading to a response of the neuron depending on SPL and FM direction. This outcome suggests that our results are dependent on the combination of the stimulus features and that further studies are needed before more general interpretations become possible.

The importance of our work lies in the fact that it is the first study in humans to probe tonotopic representation in a high-density mode. Using this mode we are able to make assertions that could not be made using a conventional approach with only 3 to 5 carrier frequencies. In addition, we expect that future parametric studies in humans will significantly expand our understanding of functional organisation of auditory representational cortex by varying stimulus features of FM-tones, as well as by continuing to explore and apply statistical procedures which can accommodate nonlinear aspects and the considerable inter-subject variability (e.g. LME).

References

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. 2 ed. Lawrence Erlbaum Associates, Hillsdale.

de Ribaupierre, F. 1997. Acoustical information processing in the auditory thalamus and cerebral cortex. In: R, R., (Ed.), The central auditory system. Oxford University Press, New York. pp. 317-383.

deCharms, R.C., Blake, D.T., Merzenich, M.M. 1998. Optimizing sound features for cortical neurons. Science 280, 1439-43.

Eggermont, J.J. 2001. Between sound and perception: reviewing the search for a neural code. Hear Res 157, 1-42.

Ehret, G. 1997. The auditory cortex. J Comp Physiol [A] 181, 547-57.

Elberling C, B.C., Kofoed B, Lebech J, Saermark K. 1982. Auditory magnetic fields: source location and 'tonotopical organization' in the right hemisphere of the human brain. Scand Audiol 11, 61-65.

Elbert, T. 1998. Neuromagnetism. In: Andrä, W., Nowak, H., (Eds.), Magnetism in medicine: a handbook. Wiley-VCH, Berlin. pp. 190-261.

Elbert, T., Sterr, A., Rockstroh, B., Pantev, C., Muller, M.M., Taub, E. 2002. Expansion of the tonotopic area in the auditory cortex of the blind. J Neurosci 22, 9941-4.

Engelien, A., Schulz, M., Ross, B., Arolt, V., Pantev, C. 2000. A combined functional in vivo measure for primary and secondary auditory cortices. Hear Res 148, 153-60.

Godey, B., Schwartz, D., de Graaf, J.B., Chauvel, P., Liegeois-Chauvel, C. 2001. Neuromagnetic source localization of auditory evoked fields and intracerebral evoked potentials: a comparison of data in the same patients. Clin Neurophysiol 112, 1850-9.

Goldstein, H. 2003. Multilevel statistical models (2nd ed.). London, Edward Arnold.

Gordon M., O'Neill W.E. 1998. Temporal processing across frequency channels by FM selective auditory neurons can account for FM rate selectivity. Hear Res 122, 97-108.

Hämäläinen, M., Hari, R., Ilmoniemi, R.J., Knuutila, J., Lounasmaa, O.V. 1993. Magnetencephalography - theory, instrumentation, and applications to noninvasive studies of the working human brain. Reviews of Modern Physics 65, 413-497.

Hartmann, W.M. 1998. Signals, sound, and sensation Springer, New York.

Heil, P., Rajan, R., Irvine, D.R. 1992a. Sensitivity of neurons in cat primary auditory cortex to tones and frequency-modulated stimuli. II: Organization of response properties along the 'isofrequency' dimension. Hear Res 63, 135-56.

Heil, P., Rajan, R., Irvine, D.R. 1992b. Sensitivity of neurons in cat primary auditory cortex to tones and frequency-modulated stimuli. I: Effects of variation of stimulus parameters. Hear Res 63, 108-34.

Heil, P., Langner, G., Scheich, H. 1992c. Processing of frequency-modulated stimuli in the chick auditory cortex analogue: evidence for topographic representations and possible mechanisms of rate and directional sensitivity. J Comp Physiol [A] 171, 583-600.

Heil, P., Irvine, D.R. 1998. Functional specialization in auditory cortex: responses to frequency-modulated stimuli in the cat's posterior auditory field. J Neurophysiol 79, 3041-59.

Horikawa, J., Nasu, M., Taniguchi, I. 1998. Optical recording of responses to frequency-modulated sounds in the auditory cortex. Neuroreport 9, 799-802.

Horikawa, J., Hess, A., Nasu, M., Hosokawa, Y., Scheich, H., Taniguchi, I. 2001. Optical imaging of neural activity in multiple auditory cortical fields of guinea pigs. Neuroreport 12, 3335-9.

Kaas, J.H., Hackett, T.A., Tramo, M.J. 1999. Auditory processing in primate cerebral cortex. Curr Opin Neurobiol 9, 164-70.

Lauter, J.L., Herscovitch, P., Formby, C., Raichle, M.E. 1985. Tonotopic organization in human auditory cortex revealed by positron emission tomography. Hear Res 20, 199-205.

Lockwood, A.H., Salvi, R.J., Coad, M.L., Arnold, S.A., Wack, D.S., Murphy, B.W., Burkard, R.F. 1999. The functional anatomy of the normal human auditory system: responses to 0.5 and 4.0 kHz tones at varied intensities. Cereb Cortex 9, 65-76.

Lütkenhöner, B., Steinstrater, O. 1998. High-precision neuromagnetic study of the functional organization of the human auditory cortex. Audiol Neurootol 3, 191-213.

Lütkenhöner, B., Krumbholz, K., Seither-Preisler, A. 2003. Studies of tonotopy based on wave N100 of the auditory evoked field are problematic. Neuroimage 19, 935-49.

Mendelson, J.R., Cynader, M.S. 1985. Sensitivity of cat primary auditory cortex (AI) neurons to the direction and rate of frequency modulation. Brain Res 327, 331-5.

Mendelson, J.R., Schreiner, C.E., Sutter, M.L., Grasse, K.L. 1993. Functional topography of cat primary auditory cortex: responses to frequency-modulated sweeps. Exp Brain Res 94, 65-87.

Nelken, I., Versnel, H. 2000. Responses to linear and logarithmic frequency-modulated sweeps in ferret primary auditory cortex. Eur J Neurosci 12, 549-62.

Nelken, I., Prut, Y., Vaadia, E., Abeles, M. 1994. In search of the best stimulus: an optimization procedure for finding efficient stimuli in the cat auditory cortex. Hear Res 72, 237-53.

Nelken, I., Versnel, H. 2000. Responses to linear and logarithmic frequency-modulated sweeps in ferret primary auditory cortex. Eur J Neurosci 12, 549-62.

Orduna, I., Mercado, E., 3rd, Gluck, M.A., Merzenich, M.M. 2001. Spectrotemporal sensitivities in rat auditory cortical neurons. Hear Res 160, 47-57.

Pantev, C., Hoke, M., Lutkenhoner, B., Lehnertz, K. 1989. Tonotopic organization of the auditory cortex: pitch versus frequency representation. Science 246, 486-8.

Pantev, C., Eulitz, C., Elbert, T., Hoke, M. 1994. The auditory evoked sustained field: origin and frequency dependence. Electroencephalogr Clin Neurophysiol 90, 82-90.

Pantev, C., Roberts, L.E., Elbert, T., Ross, B., Wienbruch, C. 1996. Tonotopic organization of the sources of human auditory steady-state responses. Hear Res 101, 62-74.

Pantev, C., Hoke, M., Lehnertz, K., Lutkenhoner, B., Anogianakis, G., Wittkowski, W. 1988. Tonotopic organization of the human auditory cortex revealed by transient auditory evoked magnetic fields. Electroencephalogr Clin Neurophysiol 69, 160-70.

Pantev, C., Bertrand, O., Eulitz, C., Verkindt, C., Hampson, S., Schuierer, G., Elbert, T. 1995. Specific tonotopic organizations of different areas of the human auditory cortex revealed by simultaneous magnetic and electric recordings. Electroencephalogr Clin Neurophysiol 94, 26-40.

Pantev C., Weisz N., Schulte, M., Elbert, T. 2003. Plasticity of the human auditory cortex. In: Pessoa, L., de Weerd, P, (Ed.), Filling-In: From Perceptual Completion to Cortical Reorganization. Oxford Univ. Press, Oxford.

Pinheiro, J.C., Bates, D.M. 2000. Mixed-effects models in S and S-Plus Springer, New York.

Romani, G.L., Williamson, S.J., Kaufman, L., Brenner, D. 1982. Characterization of the human auditory cortex by the neuromagnetic method. Exp Brain Res 47, 381-93.

Ross, B., Borgmann, C., Draganova, R., Roberts, L.E., Pantev, C. 2000. A high-precision magnetoencephalographic study of human auditory steady-state responses to amplitude-modulated tones. J Acoust Soc Am 108, 679-91.

Ross, I., Gentleman, R. 1996. R: A Language for Data Analysis and Graphics. Journal of Computational and Graphical Statistics 5, 299--314.

Schreiner, C.E., Read, H.L., Sutter, M.L. 2000. Modular organization of frequency integration in primary auditory cortex. Annu Rev Neurosci 23, 501-29.

Shamma, S.A., Fleshman, J.W., Wiser, P.R., Versnel, H. 1993. Organization of response areas in ferret primary auditory cortex. J Neurophysiol 69, 367-83.

Talavage, T.M., Ledden, P.J., Benson, R.R., Rosen, B.R., Melcher, J.R. 2000. Frequency-dependent responses exhibited by multiple regions in human auditory cortex. Hear Res 150, 225-44.

Tian, B., Rauschecker, J.P. 1994. Processing of frequency-modulated sounds in the cat's anterior auditory field. J Neurophysiol 71, 1959-75.

Tian, B., Rauschecker, J.P. 1998. Processing of frequency-modulated sounds in the cat's posterior auditory field. J Neurophysiol 79, 2629-42.

Wallace, M.N., Johnston, P.W., Palmer, A.R. 2002. Histochemical identification of cortical areas in the auditory region of the human brain. Exp Brain Res 143, 499-508.

Wessinger, C.M., VanMeter, J., Tian, B., Van Lare, J., Pekar, J., Rauschecker, J.P. 2001. Hierarchical organization of the human auditory cortex revealed by functional magnetic resonance imaging. J Cogn Neurosci 13, 1-7.

Zhang, L.I., Tan, A.Y., Schreiner, C.E., Merzenich, M.M. 2003. Topography and synaptic shaping of direction selectivity in primary auditory cortex. Nature 424, 201-5.