

Neural correlates of transmeatal cochlear laser (TCL) stimulation in healthy human subjects

Christian M. Siedentopf^{a,b,*}, Anja Ischebeck^c, Ilka A. Haala^{a,b}, Felix M. Mottaghy^d, Detlef Schikora^e, Michael Verius^a, Florian Koppelstaetter^{a,b}, Waltraud Buchberger^f, Andreas Schlager^g, Stephan R. Felber^a, Stefan M. Golaszewski^{a,b}

^a Department of Radiology II, Division of Neuroradiology, University Hospital of Innsbruck, Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria

^b fMRI-Lab, Department of Psychiatry, Medical University Innsbruck, 6020 Innsbruck, Austria

^c Department of Neurology, Medical University Innsbruck, 6020 Innsbruck, Austria

^d Department of Nuclear Medicine, University Hospital Ulm, Ulm, Germany

^e Department of Physics and Optoelectronic, University of Paderborn, Paderborn, Germany

^f Institute for Health Science, University for Health Sciences, Medical Informatics and Technology UMIT, Hall i.T., Innsbruck, Austria

^g Department of Anaesthesiology and Critical Care Medicine, Medical University Innsbruck, 6020 Innsbruck, Austria

Received 14 March 2006; received in revised form 24 July 2006; accepted 10 August 2006

Abstract

Transmeatal cochlear laser (TCL) treatment has recently been proposed as a therapeutic procedure for cochlear dysfunction such as chronic cochlear tinnitus or sensorineural hearing loss. The aim of this study was to investigate whether TLC has any influence on the central nervous system using functional MRI with healthy young adults. The laser stimulation device was placed on the tympanic membrane of both ears. A laser stimulation run and a placebo run were performed in random order. The participants were unable to differentiate between verum and placebo stimulation. In the comparison of verum to placebo runs, we observed significant activations within the left superior frontal gyrus, the right middle and medial frontal gyrus, the right superior parietal lobule, the left superior occipital gyrus, the precuneus and cuneus bilaterally, the right anterior and the left and right middle and posterior cingulate gyrus and the left thalamus. This network of brain areas corresponds well to results from previous PET studies of patients with tinnitus. Though TCL seems to have a clinically measurable effect on the central nervous system the neurophysiological mechanism leading to the observed activated neuronal network remains unknown.

© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Laser; TCL; Tinnitus; fMRI

Tinnitus is a frequent and often very disturbing phantom perception of sound as it cannot be attributed to an external source [7]. Almost 25% of a representative cohort reported to have experienced spontaneous tinnitus, 3.5% of them classified it as moderate to severe, 2% reported a severe effect on quality of life [20].

In general a dysfunction of the auditory system is assumed to be the reason for tinnitus. The pathophysiological background of tinnitus is, however, discussed controversially. Besides a peripheral damage at the level of the inner ear (cochlear dysfunction like damage of the hair cells, stereocilia decoupling, changes

in calcium ion concentration) [34], aberrant neural activity at higher levels of the auditory pathway [4,6,16] is also discussed. Most likely chronic tinnitus leads and/or is established by plastic changes in the auditory and limbic system [12].

Though the exact peripheral mechanism of tinnitus is still under debate, it is widely agreed upon that the conscious perception of tinnitus must involve the cerebral cortex [7]. Several positron emission tomography (PET) demonstrated areas subserving auditory processing, also involvement of the prefrontal cortices and limbic system in the perception of tinnitus [2,3,11,13].

Besides several standard methods, transmeatal cochlear low-level laser irradiation (TCL) [28,29] has been discussed as an alternative therapeutic procedure for cochlear dysfunction such as chronic cochlear tinnitus or sensorineural hearing loss.

* Corresponding author. Tel.: +43 512 504 23921; fax: +43 512 504 23973.
E-mail address: christian.siedentopf@fmri-easy.de (C.M. Siedentopf).

The therapeutic mechanism of this procedure is under debate. It has been assumed, that low intensity laser irradiation increases cell proliferation [5], synthesis of ATP [19] and collagen [23], the release of growth factors [10,33], promote the local blood flow in the inner ear and activates repair mechanisms in the inner ear through photochemical and photophysical stimulation of the hair-cell mitochondria [9]. The improvement rates varied from 15 to 67% of the patients, in some studies indistinguishable from placebo-effects [14,21,29,31,32].

Low-level laser therapy (LLLT) is an alternative approach to standard needle acupuncture, and has no perceivable sensory effects. In previous studies it was shown that laser acupuncture leads to activation of related cortical areas [24,25] in healthy subjects. Since TCL could have a direct effect on the inner ear, but also could involve central neural structures the aim of the present study was to determine the effect of TCL using functional magnetic resonance imaging (fMRI) in healthy volunteers. Stimulation was applied in a randomized placebo-controlled, double-blind design.

Ten healthy, right-handed volunteers (five male, five female, age 18–35 years, average age 26 ± 2.4) participated in this study that was approved by the local ethics committee. They were medication free and naive to laser stimulation or laser acupuncture. All subjects signed a written consent.

Throughout the fMRI measurements, volunteers were instructed to remain relaxed with their hands upon the abdomen, to keep their eyes closed and not to perform or focus on any specific mental activity. The light within the magnet room was dimmed and during measurement there were no other sounds besides the scanner noise. Foam padding and a special helmet fixed to the head coil was used to restrict head motions during all measurements.

A noninvasive laser-needle system (Laserneedle, Beverungen, Germany) for transmeatal cochlear laser stimulation was used. This new optical stimulation device was originally developed and build for application in acupuncture. For this study it was modified for MRI use.

The low-level-laser unit was equipped with eight identical diode lasers delivering continuous wave (CW) laserlight with a wavelength of 685 nm. This laser-needle system emits red light in CW-mode with an absolute output power of 50 mW and an effective output power of about 20 mW per laser needle, caused of the length (9 m) of the optical fibers in the fMRI environment as measured by an external power meter. The fiber core diameter was about 500 nm. The time of irradiation was 3 min \times 1 min resulting in an energy density of about 20 kJ/cm² at each tympanic membrane.

The laser irradiation of the eight laser diodes is transmitted to eight optical fibers that end in eight laser needles. For the present study four laser needles were bundled for stimulation of either side. They were inserted into a special fixation material in a specifically designed head-set so that they could be positioned into the right and left external auditory meatus without direct contact to the tympanic membrane. The laser-light was applied to the external auditory meatus by laser needles delivering a flat homogeneously illuminated circular irradiation field (Fig. 1).



Fig. 1. Fixation device for safe application of the Laserneedle® irradiation to the tympanic membrane. Laserlight within CW-mode with an effective output power of nearly 20 mW per laser needle and a wavelength of 685 nm was applied.

The laser-needle system was fully controlled by a computer using PRESENTATION Software that also synchronized laser irradiation with the MR-scanner. The computer determined the order of runs randomly and unknown to the investigators (double-blind design).

The experiment was performed on a 1.5 T whole body scanner (Magnetom VISION, Siemens, Germany) with an echo-planar capable gradient system (rise time 300 μ s, 25 mT/m) and a circular polarized head coil. For fMRI, we employed a T2* weighted single shot echo-planar sequence (repetition time: 0.96 ms, echo time: 66 ms, time resolution: 4 s, FOV: 250 mm, matrix: 64 \times 64, slice thickness: 5 mm, inter slice gap: 1.25 mm, 3.91 mm \times 3.91 mm inplane resolution, flip angle: 90°). Per image, 24 axial slices were acquired parallel to the intercommisural line. The trigger signals from the scanner were recorded to the nearest millisecond on a separate computer and were used to control stimulus presentation delivered by the laser-needle system. The first five images of each functional measurement were discarded from the analysis to allow for magnetic saturation.

A double-blinded, placebo controlled cross-over design was used. An experimental session consisted of two fMRI measurements: One run applying placebo stimulation (no stimulation-laser diodes turned off) and another run with laser stimulation. The order of the two runs and the switching on/off of the laser canals were chosen randomly by the computer and was unknown to the experimenter and the participant. The runs were acquired with a break of 10 min in between. The laser needles were placed before the first run and kept in exactly the same position during both conditions resulting in the same tactile stimulus in both runs. During the laser stimulation the laser light was alternately switched on and off in a typical fMRI block design. For the duration of 15 fMRI images the laser light was alternately switched on (condition A) and off (condition R) controlled by the computer. The measurement on a single image took 4 s. A total amount of 90 images was acquired (duration: 6 min, condition sequence: RARARA). Five dummy images were acquired prior

Table 1
Activation clusters detected for the laser stimulations group results

| Anatomical Location | | Brodmann area | <i>p</i> -Value | <i>T</i> -value | Cluster-size | MNI-coordinates of max value | | |
|---------------------|---------------------------|---------------|-----------------|-----------------|--------------|------------------------------|----------|----------|
| | | | | | | <i>x</i> | <i>y</i> | <i>z</i> |
| R L | Precuneus | 7 | 0.000 | 5.96 | 273 | 8 | −68 | 44 |
| R L | Cuneus | 31 | | | | | | |
| L | Superior occipital gyrus | 19 | | | | | | |
| R | Superior parietal lobule | 7 | | | | | | |
| R L | Posterior cingulate gyrus | 23 | | | | | | |
| R L | Middle cingulate gyrus | 23 | 0.001 | 4.77 | 60 | −36 | −68 | −24 |
| L | Thalamus | | | | | | | |
| R | Medial frontal gyrus | 8/9 | 0.028 | 4.75 | 30 | 8 | 32 | 36 |
| R | Middle cingulate gyrus | 32 | | | | | | |
| R | Anterior cingulate gyrus | 32 | | | | | | |
| L | Superior frontal gyrus | 10 | 0.034 | 4.30 | 28 | −32 | 60 | 8 |
| R | Middle frontal gyrus | 8/9 | 0.048 | 3.85 | 25 | 44 | 20 | 40 |

Activations are reported for clusters which surpassed an initial threshold of $p < 0.001$ uncorrected and had a corrected p -value of $p < 0.05$ on cluster level.

to the experimental time series to allow for magnetic saturation. They were discarded from the analysis.

After each run subjects were asked to guess whether they just received placebo or verum TCL. They stated that both runs were indistinguishable for them. There were no feelings of heat or anything else, or cues by sound or light accompanying the stimulation. To investigate the ability of subjects to perceive the presence of stimulation, an additional behavioral experiment with 10 volunteers (five male, five female, average age 26 ± 2.4) outside the scanner was conducted. The laser was put to the tympanic membrane of each ear in the same way like the fMRI experiment and stimulation was randomly applied (25 times on, 25 times off in total per subject). The experimenter was seated behind the subject and controlled the stimulation. The participants were told that laser stimulation was switched on in half of the trials and that they should guess either way, even if they were unable to feel anything. No participant was able to indicate the presence or absence of laser stimulation significantly above guessing probability (binomial test).

Data analysis was performed using SPM99 (The Wellcome Department of Cognitive Neurology, London; <http://www.fil.ion.ucl.ac.uk/spm/>). The functional images of each participant were realigned, coregistered to the individual high-resolution anatomical scan and finally normalized into a standard stereotactic space [27]. The coordinates given by SPM99 were corrected to correspond more closely to the atlas of Talairach & Tournoux [27] using the transformation algorithm by M. Brett (<http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml>).

The functional data were finally smoothed with a Gaussian kernel of 8 mm FWHM. The delta function of stimulus onsets for each condition was convolved with the canonical form of the hemodynamic response function (HRF) as defined in SPM99 and its first temporal derivative (HRF'). Statistical inferences were drawn on the basis of the general linear model as it is implemented in SPM. Linear contrasts were calculated for the comparisons between conditions. Activations are reported for clusters which surpassed an initial threshold of $p < 0.001$ uncorrected for multiple comparisons and had a corrected p -value of $p < 0.05$ on cluster level.

Due to the small number of subjects a fixed effects analysis was used rather than a random effects analysis. It should be noted, however, that results from a fixed effects analysis only relate to the sample being tested and do not allow generalization to the population.

The group analysis for the laser stimulation versus rest shows five activation clusters (see Table 1). Significant activations were found within the left superior frontal gyrus (Brodmann area BA 10), the right middle and medial frontal gyrus (BA 8/9), the right superior parietal lobule (BA 7), the left superior occipital gyrus (19), the left and right precuneus and cuneus (BA 7/31), the right anterior cingulate gyrus (BA 32), the middle and posterior cingulate gyrus (BA 23/32) and the left thalamus (Figs. 2 and 3).

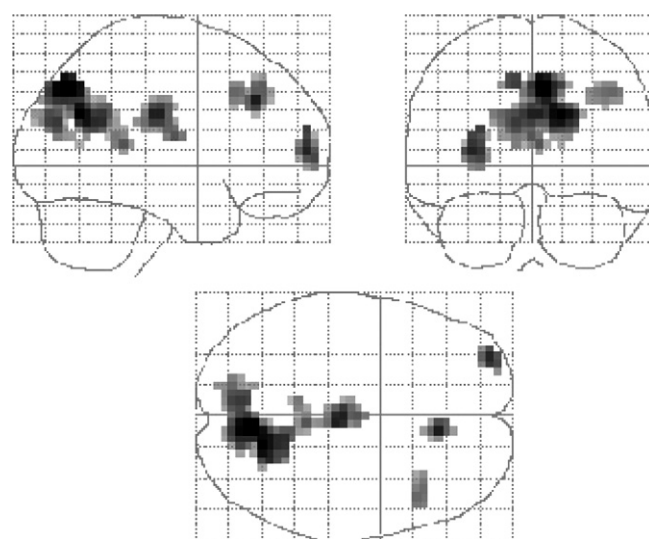


Fig. 2. Glass brain image of the fMRI group data ($n = 10$) for laser irradiation of the tympanic membrane showing prefrontal, parietal, occipital and limbic activation foci. Clusters are reported as being significant if they passed an uncorrected threshold of $p < 0.001$ with a corrected p -value on cluster-level of less than 0.05, corrected for multiple comparisons.

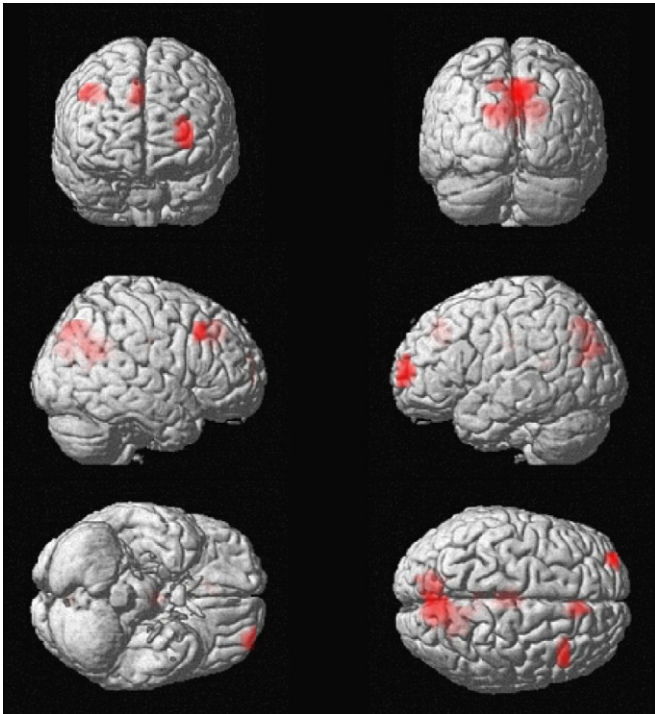


Fig. 3. Surface rendering of the fMRI group results ($n = 10$) for laser irradiation of the tympanic membrane.

The analysis placebo versus rest revealed no significantly activated clusters. Furthermore, the analyses rest versus laser stimulation and rest versus placebo showed no significant activation.

A thalamo-limbic-fronto-parietal network was activated by TCL. This corresponds well to the network of brain areas reported in previous studies of tinnitus [11,13–15,26]. The majority of activation foci were in the right hemisphere, in accordance with the results of Mirz and co-workers [13]. In patients with tinnitus additionally the auditory cortex is activated. It is suggested that the perception of tinnitus is mediated by a specific neural network [12,30].

According to the model of central tinnitus generation from Mahlke and Wallhäusser-Franke [12] the induction of a peripheral hearing deficit on the auditory system is the primary and specific effect. The auditory system tries to compensate for the loss of input which results in an increased and constant cortical activation within the auditory cortex (AC) that is not triggered by an auditory input signal [4,11]. The result is the perception of “ringing in the ears” in absence of an external auditory source.

The persistence of the enhanced activation in AC or at the level of the dorsal cochlear nucleus leads to plastic changes in the related neural network [17,18]. Plasticity is known to be fostered by activation of the limbic system [12], which is part of the network seen in patients and in the current study as well. Thalamic and cortical connections from the limbic system facilitate the tinnitus signal in evoking an emotional and sympathetic autonomic response to the tinnitus. Memory, which relates the stimulus to previous experiences, may play an important role in this process, too. Therefore, stressors that activate the limbic system, and moreover coincide in time with aberrant activity within

the central auditory system, are likely to increase excitability and plasticity in AC, and thus are likely to consolidate a tinnitus.

The mechanism leading to the observed activated neuronal network by means of TLC is vague. It cannot be explained by a supra-sensory stimulation of the tympanic membrane, since subjects were not able to differentiate between real and placebo stimulation. The important point that is stressed in the introduction is the clinically measurable effect. To date the physiological mechanisms are completely unclear. In this pilot study we were interested to test the hypothesis that TLC has not only a local but a central effect.

TLC could lead to a break-down of the pathological enhanced activity within the tinnitus related neuronal network by means of overriding pulsed activations at the level of AC or the dorsal cochlear nucleus. This is certainly highly speculative, however other therapeutic approaches or interferences with tinnitus rely probably on comparable mechanisms. It is possible to interfere with tinnitus setting a virtual lesion in the temporoparietal cortex with high-frequency transcranial magnetic stimulation [22]. On the other side behavioral therapeutic approaches [1,8] or simply masking noises have probably comparable effects.

In conclusion the neuronal correlates of TCL were investigated for the first time. The results demonstrate central activations in brain regions which play an important role in the development and consolidation of tinnitus. While TCL has a clinically measurable effect the mechanism leading to the observed activated neuronal network is still unknown. The fact that TCL interferes with cortical areas could potentially lead to new treatment approaches.

Acknowledgment

The authors wish to express their gratitude to Dr. Sandi Suwanda, for helpful discussions on clinical application of laser treatment in tinnitus patients and to Mathias Wiedemann for technical support.

The work was supported by the Biomed2 Project Grant PL 950870 of the European Community.

References

- [1] G. Andersson, Psychological aspects of tinnitus and the application of cognitive-behavioral therapy, *Clin. Psychol. Rev.* 22 (2002) 977–990.
- [2] A.T. Cacace, J.P. Cousins, S.M. Parnes, D.J. McFarland, D. Semenov, T. Holmes, C. Davenport, K. Stegbauer, T.J. Lovely, Cutaneous-evoked tinnitus. Review Of neuroanatomical, physiological and functional imaging studies, *Audiol. Neurootol.* 4 (5) (1999) 258–268.
- [3] A.L. Giraud, S. Chery-Croze, G. Fischer, C. Fischer, A. Vighetto, M.C. Gregoire, F. Lavenne, L. Collet, A selective imaging of tinnitus, *Neuroreport* 10 (1) (1999) 1–5.
- [4] G. Gerken, Central tinnitus and lateral inhibition: An auditory brainstem model, *Hear. Res.* 97 (1996) 75–83.
- [5] H.F.I. Hans, V. Breugel, D. Bar, Power density and exposure time of He–Ne laser irradiation are more important than total energy dose in photobiomodulation of human fibroblasts in vitro, *Lasers Surg. Med.* 12 (1992) 528–537.
- [6] P.J. Jastreboff, J.F. Brennan, C.T. Sasaki, An animal model for tinnitus, *Laryngoscope* 98 (1988) 280–286.
- [7] P.J. Jastreboff, Phantom auditory perception (tinnitus): Mechanisms of generation and perception, *Neurosci. Res.* 8 (1990) 221–254.

- [8] P.J. Jastreboff, M.M. Jastreboff, Tinnitus retraining therapy for patients with tinnitus and decreased sound tolerance, *Otolaryngol. Clin. N. Am.* 36 (2003) 321.
- [9] T. Karu, Molecular mechanism of the therapeutic effect of low-intensity laser radiation, *Lasers Life Sci.* 2 (1988) 53–74.
- [10] N. Kipshidze, V. Nikolaychik, M.H. Keelan, L.R. Shankar, A. Khanna, R. Kornowski, M. Leon, J. Moses, Low-power Helium: Neon laser irradiation enhances production of vascular endothelial growth factor and promotes growth of endothelial cells in vitro, *Lasers Surg. Med.* 28 (2001) 355–364.
- [11] A.H. Lockwood, R.J. Salvi, M.L. Coad, M.L. Towsley, D.S. Wack, B.W. Murphy, The functional neuroanatomy of tinnitus: Evidence for limbic system links and neural plasticity, *Neurology* 51 (2) (1998) 647–648.
- [12] C. Mahlke, E. Wallhauser-Franke, Evidence for tinnitus-related plasticity in the auditory and limbic system, demonstrated by arg3.1 and c-fos immunocytochemistry, *Hear Res.* 195 (1–2) (2004) 17–34.
- [13] F. Mirz, B. Pedersen, K. Ishizu, P. Johannsen, T. Ovesen, H. Stodkilde-Jorgensen, A. Gjedde, Positron emission tomography of cortical centers of tinnitus, *Hear Res.* 134 (1–2) (1999) 133–144.
- [14] F. Mirz, R. Zachariae, S.E. Andersen, A.G. Nielsen, L.V. Johansen, P. Bjerring, C.B. Pedersen, The low-power laser in the treatment of tinnitus, *Clin. Otolaryngol.* 24 (1999) 346–354.
- [15] F. Mirz, A. Gjedde, H. Sodkilde-Jrgensen, C.B. Pedersen, Functional brain imaging of tinnitus-like perception induced by aversive auditory stimuli, *Neuroreport* 11 (3) (2000) 633–637.
- [16] A.R. Møller, Similarities between chronic pain and tinnitus, *Am. J. Otol.* 18 (1997) 577–585.
- [17] A.R. Møller, Pathophysiology of tinnitus, *Otolaryngol. Clin. N. Am.* 36 (2003) 249.
- [18] W. Mühlnickel, T. Elbert, E. Taub, H. Flor, Reorganization of auditory cortex in tinnitus, *Proc. Natl. Acad. Sci. USA* 95 (1998) 10340–10343.
- [19] S. Passarella, E. Casamassima, S. Molinari, E. Pastore, E. Quagliariello, I.M. Catalano, A. Cingolani, Increase of proton electrochemical potential and ATP synthesis in rat liver mitochondria irradiated in vitro by helium–neon laser, *FEBS Lett.* 175 (1984) 95–99.
- [20] M. Pilgramm, R. Rychlik, H. Lebisch, H. Siedentopf, G. Goebel, D. Kirchhoff, Tinnitus in der Bundesrepublik Deutschland—eine repräsentative epidemiologische Studie, *HNO aktuell* 7 (1999) 261–265.
- [21] P. Plath, J. Olivier, Results of combined low-power laser therapy and extracts of *Ginkgo biloba* in cases of sensorineural hearing loss and tinnitus, *Adv. Otorhinolaryngol.* 49 (1995) 101–104.
- [22] C. Plewnia, M. Bartels, C. Gerloff, Transient suppression of tinnitus by transcranial magnetic stimulation, *Ann. Neurol.* 53 (2) (2003) 263–266.
- [23] G.K. Reddy, L. Stehno-Bittel, C.S. Enwemeka, Laser photostimulation of collagen production in healing rabbit achilles tendons, *Lasers Surg. Med.* (1998) 281–287.
- [24] C.M. Siedentopf, S.M. Golaszewski, F.M. Mottaghy, C.C. Ruff, S. Felber, A. Schlager, Functional magnetic resonance imaging detects activation of the visual association cortex during laser acupuncture of the foot in humans, *Neurosci. Lett.* 327 (1) (2002) 53–56.
- [25] C.M. Siedentopf, F. Koppelstaetter, I.A. Haala, V. Haid, P. Rhomberg, A. Ischebeck, W. Buchberger, S. Felber, A. Schlager, S.M. Golaszewski, Laser acupuncture induced specific cerebral cortical and subcortical activations in humans, *Lasers Med. Sci.* 20 (2) (2005) 68–73.
- [26] A. Shulman, M. Afriyie, F. Aronson, W. Abel, B. Goldstein, SPECT imaging of brain and tinnitus—neurotologic/neurologic implications, *Int. Tinnitus J.* 1 (1995) 13–29.
- [27] J. Talairach, P. Tournoux, *Stereotaxic Atlas of the Human Brain*, Georg Thieme Verlag, New York, 1988.
- [28] S. Tauber, R. Baumgartner, K. Schorn, W. Beyer, Lightdosimetric quantitative analysis of the human petrous bone: Experimental study for laser irradiation of the cochlea, *Lasers Surg. Med.* 28 (2001) 18–26.
- [29] S. Tauber, K. Schorn, W. Beyer, R. Baumgartner, Transmeatal cochlear laser (TCL) treatment of cochlear dysfunction: A feasibility study for chronic tinnitus, *Lasers Med. Sci.* 18 (3) (2003) 154–161.
- [30] E. Wallhäuser-Franke, G. Langner, Phantom sounds: Mechanisms of tinnitus in the central nervous system, *Neuroreport* 1/01 (2001) 21–27.
- [31] H. Wedel, L. Calero, M. Walger, S. Hoenen, D. Rutwalt, Soft laser/Ginkgo therapy in chronic tinnitus. A placebo-controlled study, *Adv. Otorhinolaryngol.* 49 (1995) 105–108.
- [32] L. Wilden, D. Dindinger, Treatment of chronic complex diseases of the inner ear with low-level laser therapy (LLLT), *Laser Therap.* 8 (1996) 209–212.
- [33] W. Yu, J.O. Naim, R.J. Lanzafame, The effects of photoirradiation on the secretion of TGF- β , PDGF and bFGF from fibroblasts in vitro, *Lasers Surg. Med. [Suppl.]* 6 (1994) 8.
- [34] H.P. Zenner, A. Ernst, Cochlear-motor, transduction and signaltransfer tinnitus: Models for three types of cochlear tinnitus, *Eur. Arch. Otorhinolaryngol.* 249 (1993) 447–454.