The Influence of Didlofenac and Ketoprofen of Function of Heart and Liver

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Introduction
According to new references, there is connection between non-steroidal anti-inflammatory drugs and oxidative stress and increasing risk of myocardial infarction. The aim of this research was to establish are diclofenac and ketoprofen contributing to oxidative stress and their influence on myocardial function during intra venous infusion of barium-chloride and strophantin.

Methods
We used Wistar rats, divided in 3 groups: control group (which didn’t take any xenobiotic), diclofenac (8 mg/kg/day, per os) and ketoprofen (8 mg/kg/day, per os) treated group. After this treatment, experimental groups were divided into 2 subgroups. The first subgroup of both groups got 6% solution of barium-chloride (BaCl2), and the second 1.5 mg/ml strophantin solution by continuous intravenous infusion. During these infusions, we followed primary rhythm changes, tachyarrhythmia and cardio toxic effects on ECG. Doses of BaCl2 and strophantin that was necessary to induce these rhythm changes were calculated and levels of indicators of oxidative stress were measured in liver homogenate.

Results
Pretreatment with diclofenac and ketoprofen didn’t change significantly influence of barium-chloride on myocard function. Dose of strophantin that was needed to induce first rhythm change was significantly lower in ketoprofen treated group comparing with control (0.331 mg : 0.53 mg). Pretreatment with diclofenac and ketoprofen caused statistically significant decrease of catalase activity, comparing with control (5.85 : 6.06 : 9.02). Furthermore, diclofenac and ketoprofen didn’t change lipide peroxidation level and reduced gluthation concetration, comparing with control.

Discussion/Conclusion
We can conclude that diclofenac and ketoprofen change effects of cardioactive substances, but in used doses do not induce oxidative stress.

Topically-used Gentamicin Attached to Nanofibre Micro-Dispersed Oxidised Cellulose Compared with Gentamicin Attached to Collagen Foam in an Acute Wound Infection Model
An Experimental Study

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Introduction
The aim was to examine the effect of topically-used gentamicin attached to a carrier, micro-dispersed oxidised cellulose (MDOC) in nanofibre form, in acute wound infection treatment and to compare it with gentamicin attached to collagen foam.
Methods
Twelve female domestic swines were used in a model of a full-thickness infected dermal wound. The effectiveness of both the materials in infections caused by Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli was tested.

Results
The effectiveness of both gentamicin with MDOC and gentamicin with collagen was comparable in Pseudomonas aeruginosa and Escherichia coli infections according to microbiological findings. With Staphylococcus aureus infections was a significantly higher percent of negative cultures if MDOC with gentamicin was administered. When macroscopically assessed, 100% of infected wounds treated by gentamicin attached to MDOC were without signs of local infection compared with only 16.7% when gentamicin attached to collagen foam was used.

Discussion/Conclusion
When combined with a nanofibre MDOC carrier, topically-used gentamicin seems to be rendered more for treatment of full-thickness skin infections. The positive influence of MDOC on healing process of dermal wound was shown and a resulting good haemostatic effect confirmed.

Acknowledgments
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Silicotuberculosis
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Introduction
Silicosis is a parenchymal lung disease caused by inhalation of crystalline silica, a component of rock and sand. Fibrinogenic dust causes non-specified inflammatory reaction on the level of terminal airways. Fibrous transformation of lung interstitium and cicatrices of lungs. Irreversible changes are being created which continue even after the risk is over. Professions at risk: tunnelers, quarry works, sandblasters, stonecutters, glassworks, potteries, construction works – white masons.

Methods
RTG lung pictures First stage: dust stigmatisation, strengthen bronchovascular study Second stage: reticulosis, dense rete of minor opacities Simple silicosis: generalised nodulation, round opacities of about 1,5mm-10mm in middle lung zones Complicated silicosis: opacity bigger than 10mm in upper and middle lung zones, exercion dyspnoea, later dormancy, productive cough, restricted or combined ventilation defect Silicotuberculosis: frequent complication

Results
Casuistic s of the patient: Pacient Š.K. born 1928 In 1972 simple pneumoconioses as a vocational disease was reported. The patient worked as a miner in the Mine Dobre Štestí (Good Luck) in Dobrany. In the period from 22.9. 1975 up to 4.1. 1976 he was hospitalised on the lung department of the hospital in Klatovy for dg. focal tuberculosis of lungs. Following an antituberculosis treatment, which continued in an ambulatory way, a little regression took place in rtg picture and the patient was transferred to the surface of the mine as a engineer. In 1975 notification of inactive silicotuberculosis. During his hospitalisation of the lung department in Klatovy, the activity of the procedure during the antituberculosis treatment which lasted until march 1977 and the disease was reported as active silicotuberculosis.

Discussion/Conclusion
The patient is in permanent dispensory care of the Clinics of occupational medicine in Plzen where he regularly every year goes to an investigation of health state and control RTG of lungs.
Adiponectin Concentrations During Pregnancy and at Delivery and their Relationship with Antropometrical Data in Newborns and Children

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Introduction
Our aim was to investigate the relationship between maternal and cord blood plasma adiponectin and anthropometric indices measured at birth and 4 years of age.

Methods
We measured plasma adiponectin concentrations by ELISA in a subgroup of healthy mothers and their newborns participated in a supplementation study (n=81) at the 20th, 30th week of gestation, and at delivery.

Results
Plasma adiponectin concentrations significantly decreased during pregnancy (20th week: 13.19 [6.24]; 30th week: 10.83 [5.18]; delivery: 10.15 [4.56]; µg/ml, mean [SD], p<0.05) and were about 3-fold higher in venous cord blood (35.33 [13.89]) than in maternal blood. There were significant positive correlations between adiponectin concentrations in cord blood and both triceps skinfold (r=0.378, p<0.01) and subscapular skinfold (r=0.410, p<0.01) in newborns at birth. In contrast, at 4 years of age we found significant positive correlations between, on the one hand, chest and waist circumference and, on the other hand, maternal adiponectin concentrations at the 20th week (r=0.311, p<0.05 and r=0.370, p<0.01) and 30th week of gestation (r=0.378, p<0.01 and r=0.388, p<0.01) and at delivery (r=0.317, p<0.05 and r=0.424, p<0.01).

Discussion/Conclusion
Anthropometric data measured at birth may be related to the adiponectin producing capacity of the newborn, whereas maternal adiponectin may have a programming effect on later growth of the children.

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Effect of Celiac Disease and Type-1 Diabetes Mellitus on Fatty Acid Composition of Plasma Phospholipids in Children
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Introduction
To investigate the availability of long-chain polyunsaturated fatty acids (LCPUFA) in children with celiac disease (CD) with and without type-1 diabetes mellitus (DM).

Methods
Fatty acid composition of plasma phospholipids was determined in 20 children with CD (age: 13.1 ± 3.8 years, mean ± SD), in 8 children with CD and DM (CDDM, age: 11.6 ± 4.0 years) and in 21 healthy controls (age: 13.3 ± 3.7 years).

Results
There were no significant differences between controls and children with CD only in any of the investigated LCPUFA. In contrast, values of linoleic acid were significantly higher [19.68 (2.53) vs. 22.98 (4.88); median (IQR), p < 0.05], whereas those of dihomo-γ-linolenic acid were significantly lower [3.43 (0.96) vs. 2.42 (0.85), p < 0.01] in children with CDDM than in controls. Similarly, we found significantly lower values of eicosapentaenoic acid [0.24 (0.10) vs. 0.15 (0.15), p < 0.01] in children with CDDM than in healthy controls. Values of docosahexaenoic acid were also significantly lower [3.03 (0.49) vs. 2.32 (0.71), p < 0.05] in children with CDDM than in children with CD.
Discussion/Conclusion

1.) In this study, CD on its own did not influence the availability of polyunsaturated fatty acids in children. 2.) In contrast, we found significantly lower LCPUFA values in children with CDDM than in healthy controls. 3.) Children with CDDM may benefit from an enhanced dietary supply of LCPUFA.

VENCEDOR:

Identification of a CYP27A1 Splice Mutation in a Family Presenting With Pulverulent Cataracts and Developmental Delay

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Introduction
A consanguineous Bangladeshi family with three children with pulverulent cataract and global developmental delay were ascertained. Learning difficulties were also present in the mother and two additional siblings without cataract. At this stage it was unclear whether the developmental delay and cataracts were linked to a single condition (with variable expression) or two different disorders.

Methods
Genetic linkage studies, employing an autozygosity mapping approach, were undertaken to investigate the genetic basis of cataracts in the family. Genome-wide linkage analysis using a 10K Affymetrix SNP Microarray for two of the children with cataracts was used.

Results
Six regions of extended homozygosity (>2Mb) were identified. One of these regions, a 3.6Mb interval on chromosome 2 (218.99-222.59 Mb), contained the crystallin CRYBA2 gene. Sequencing of the CRYBA2 did not reveal a germline mutation but analysis of CYP27A1 (which maps to the same region) demonstrated that all the children with cataracts were homozygous for a germline intron 6 CYP27A1 splice site mutation (G→A at +1).

Discussion/Conclusion
The diagnosis of cerebrotendinous xanthomatosis (CTX) was confirmed by biochemical analysis. Other features of CTX were identified in affected children. Early diagnosis of CTX is important as treatment with chenodeoxycholic acid, with or without HMG-CoA inhibitors, corrects biochemical abnormalities and arrests, and possibly reverses disease progression. If suspected, CTX can be diagnosed by the determination of the serum cholestanol level. Unusually, in our case the diagnosis was made in the absence of xanthomas and with the assistance of molecular genetic testing. Childhood cataract is a rare but previously reported presentation of CTX.

Funding
Fight For Sigh

Oncology & Molecular Biology

Human γδ T cells – A Lymphoid Lineage Cell Capable of Professional Phagocytosis
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Introduction
Innate immune cells express hard programmed receptors that sense molecular structures on microbes and altered
self-cells which enable the uptake of these agents as a broad first-line defense. Adaptive immune cells evolve clonally within an individual by somatic rearrangement providing a delayed but tailored response. Transfer of information from the initial innate contact to the adaptive immune system via antigen acquisition and presentation is central to an effective adaptive immune response. Myeloid cells such as monocytes, macrophages, neutrophils and myeloid dendritic cells clearly display innate characteristics, whilst lymphoid lineage B and γδ T cells represent the classical adaptive effector responses. γδ T cells, however, whilst sharing γδ T cell functions, also perform immune surveillance of an innate character.

Methods
Flow cytometry Immunofluorescence and confocal microscopy Electron microscopy Antigen presentation assays

Results
Here we demonstrate, for the first time, that γδ T cell are capable of phagocytosis via antibody opsonization and CD16 (FcγRIII), leading to antigen processing and presentation on MHC class II.

Discussion/Conclusion
These findings of distinct myeloid characteristics in γδ T cells strongly support the suggestion that γδ T cells are evolutionarily ancient lymphocytes and have implications for our understanding of their role in transitional immunity and the control of infectious diseases and cancer.

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Stat3 Protects from Liver Injury and Fibrosis in a Mouse Model of Sclerosing Cholangitis

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Introduction
Signal transducer and activator of transcription 3 (Stat3) is the main mediator of interleukin-6 type cytokine signaling required for hepatocyte proliferation and hepatoprotection but its role in sclerosing cholangitis (SC) and other cholestatic liver diseases remains unresolved.

Methods
We investigated the role of Stat3 in inflammation-induced cholestatic liver injury and used mice lacking the multidrug resistance gene 2 (mdr2-/-) as a model for SC.

Results
We demonstrate that conditional inactivation of stat3 in hepatocytes and cholangiocytes (stat3Dhc) of mdr2-/- mice strongly aggravated bile acid-induced liver injury and fibrosis. Similarly, stat3Dhc mice are more sensitive to cholic acid feeding than control mice. Global gene expression analysis demonstrated that hepatoprotective signals via epidermal growth factor and insulin-like growth factor 1 are affected upon loss of Stat3.

Discussion/Conclusion
Our data suggest that Stat3 protects cholangiocytes and hepatocytes from bile acid-induced damage thereby preventing liver fibrosis in cholestatic diseases.
Biological Evaluation of Exemestane, an Aromatase Inhibitor, in an Aromatase Expressing MCF-7aro Breast Cancer Cell Line

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Introduction
Estrogens are pivotal in the growth and development of neoplastic mammary tissue. Nowadays, there are several therapeutic approaches to block estrogen actions. One is based on the inhibition of aromatase, the enzyme responsible for catalyzing the conversion of androgens to estrogens. Exemestane, a synthetic steroidal aromatase inhibitor (AI) is an effective alternative to the classical treatment with tamoxifen, for post-menopausal women with ER-positive breast cancer [1]. However, its mechanism of action in cells is not totally understood neither is the eventual development of resistance in some patients.

Methods
We have previously used an ER-positive aromatase-overexpressing breast cancer cell line (MCF-7aro) for the study of antiaromatase activity of newly synthesized AIs [2,3]. In this work we evaluated the in vitro effects of exemestane, in cell viability and proliferation and cell cycle progression. Cells were cultured in steroid-free medium and treated with exemestane for different times (3-9 days) and concentrations (2,5 µM- 15 µM). MTT, LDH, thymidine incorporation and cell cycle assays were performed and morphologic alterations were analysed by Giemsa and Hoechst staining.

Results
The results obtained showed that cell viability decreased in a time- and dose-dependent manner after hormone (1nM testosterone)-stimulated proliferation. The antiproliferative effect was accompanied by morphologic alterations (blebs formation, chromatine condensation and fragmentation).

Discussion/Conclusion
The results suggest that MCF-7aro could be used as an in vitro model of aromatase-driven breast cancer for evaluation of AIs used in hormonal therapy and may help in the elucidation of their mechanisms of interaction with breast cancer cells, allowing the finding of more potent and selective endocrine therapies.

REFERENCES

8473T>C COX-2 Polymorphism and Susceptibility for Lung Cancer
A Potential Biomarker

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Introduction
Lung cancer (LC) presents a major health problem in the world, being the most common cause of death from cancer. Cyclooxygenase-2 (COX-2), normally undetected in physiological conditions, is promptly triggered under inflammatory and tumor promotion settings, contributing to key steps of carcinogenesis. Up-regulation of COX-2 is an early event in lung carcinogenesis. It is known to be induced by cigarette smoke condensate in vitro and by the tobacco-specific carcinogen nitrosamine 4-(methylNitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in mice. The 8473T>C COX-2 polymor-
phism in an AU-rich elements region (3'UTR) might contribute to cancer development by influencing COX-2 mRNA stability. The aim of our study was to assess the influence of this polymorphism in the development of LC.

**Methods**

This case-control study gathered 1069 individuals: 718 healthy individuals and 351 patients with histopathologically confirmed lung cancer, from the Northern region of Portugal. The 8473T>C COX-2 polymorphism genotypes were determined by Real-Time PCR allelic discrimination technique.

**Results**

We found no statistically significant differences in the distribution of the 8473T>C polymorphism genotypes between LC cases and controls (P=0.122). However, in a stratified analysis by histological type and gender we observed an increased risk for epidermoid non-small cell lung cancer (OR=1.47; 95%CI:1.00-2.15) and more interesting, males C allele carriers revealed a higher susceptibility for epidermoid non-small cell lung cancer (OR=1.55; 95%CI:1.03-2.33).

**Discussion/Conclusion**

The 8473T>C COX-2 polymorphism appears to modulate the genetic susceptibility for epidermoid non-small cell lung cancer, especially in males. This genetic profiling based higher-risk group definition may help in the identification of individuals with higher susceptibility for LC development.

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**Oral Cancer Therapy**

*Propose of NNGH and EGCG as New Agents*

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**Introduction**

Oral squamous cell carcinoma is one of the major health-care dilemmas. Understanding the underlying molecular pathogenesis of this disease may afford new opportunities for future treatments. It is currently accepted that deregulated cell proliferation and apoptosis and cell adhesion lead to development of cancer. Therefore it seems that exploiting the apoptotic potential of cancer cells might lead to new therapies that could be less toxic. The aim of this study is to evaluate the potential therapeutic of the metalloproteinase inhibitor, N-Isobutyl-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH) and of the polyphenolic compound, Epigallocatechin-3-Gallate (EGCG), alone and in association with conventional anticarcinogenic agent, cisplatin, in oral cancer.

**Methods**

For this purpose human oral cancer cell line (HSC-3) was incubated with increasing concentrations of NNGH and EGCG, during 72 hours. Cell viability was estimated by alamar blue test. Cell death was evaluated by annexin V/propidium iodide incorporation and detected by flow cytometry and cell morphology was evaluated by light microscopy examination of May-Grünwald Giemsa stained cells.

**Results**

Preliminary results show that NNGH (250µM) and EGCG (150µM) as a single agents induced a diminution of cell proliferation in a time dependent manner with an IC50 of 250µM and 150µM, respectively. When in association, these two agents demonstrate a synergistic effect, as well as when in association with cisplatin.

**Discussion/Conclusion**

These results suggest that NNGH and EGCG may be used as potential therapeutic approach in oral cancer in monotherapy and in association with convencional anticarcinogenic agent.
Effect of MIR-34 Family in the Radiosensitivity of Non-Small Cell Lung Cancer Cell Lines

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Introduction
MicroRNAs have been identified as important players in carcinogenesis and as potential modulators of resistance to cytotoxic therapy. The miR-34 family, known to have important roles in the regulation of cell proliferation and in P53-dependent apoptosis, has a reduced expression in lung cancer. This work aimed to verify whether the overexpression of miR-34b could reduce resistance to radiation in non-small cell lung cancer cell lines and to investigate the mechanisms involved.

Methods
Radioresistant lung cancer cell lines A549 and H1299 were treated with increasing doses of radiation (4Gy, 8GY and 12Gy) 24 h after transfection with pre-miR-34b or a pre-miR control. miR-34 family members expression levels were evaluated by real time RT-PCR. Cell viability was assessed by a clonogenic survival test. The involvement of apoptosis was evaluated by flow cytometry using annexinV/propidium iodide and by the expression modulators, namely BAX, BCL2 and P53 using monoclonal antibodies.

Results
miR-34b was the miR-34 family member with the lowest levels of expression in the two cell lines. However, clonogenic survival test showed an increased sensitivity to radiation in both cell lines transfected with pre-miR-34b. On the other hand, in irradiated A549 cell line we observed only slightly increased in P53 expression. We don’t found any evidence of apoptotic effect 48h after cell irradiation, with or without transfection.

Discussion/Conclusion
Our preliminary results suggest that overexpression of miR-34b can circumvent resistance to radiation in non-small cell lung cancer cell lines. This effect seems to be independent of apoptotic mechanisms.

BREAST CANCER PROTEOMIC BIOMARKER DISCOVERY

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Introduction
Molecular diagnostics classifying patients into risk, treatment and response specific sub groups are the focus of cancer biomarker discovery 1-2. To date, early disease and relapse detection and molecular pathway understanding hold the best promise towards improved breast and other anticancer strategies. Here, we present our findings in breast cancer proteomics.

Methods
Serum samples were prefractionated using strong anion exchange resins and pH gradient elution. Robotic handling was used to minimise pre analytical bias. Samples were prepared using a low stringency binding buffer and weak cation exchange arrays and bean columns. We analysed 347 serum samples (210 healthy, 73 breast cancer and 64 benign breast disease) using MS. Putative proteomic peaks were validated using an independent set and identical protocols. Mann-Whitney U test and CART analysis were adopted to identify differential expression and classification rules. SDS PAGE was used for protein purification and MALDI TOF/TOF is adapted for bona fide biomarker identification.

Results
Differentially expressed candidate biomarkers (p≤0.05) were reported and validated between groups. 9 differentially expressed peaks were detected within the cancer and healthy control groups. In addition, 11 peaks differentiated the
cancer and benign disease group. Finally, 7 candidate biomarkers showed differential patterns between the healthy and benign breast disease group.

**Discussion/Conclusion**
Identification of these biomarkers is ongoing and will be followed by further immuno assay validation (WB, ELISA) and molecular pathway and network mapping. This would add more molecular evidence in translational breast cancer research.

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**Angiogenic and Inflammatory Activities Modulated by Beer Polyphenols**
*Taking Skin-wound Healing as a Model*

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**Introduction**
Angiogenesis, is deregulated in pathologies like cancer, diabetes and cardiovascular diseases. Hops used in beer production are a source of polyphenols such as xanthohumol (XN), and its metabolites isoxanthohumol (IXN) and 8-prenylxanthohumol (8PN), known to influence inflammation and angiogenesis.

**Methods**
For skin wound-healing assay, two skin-thickness longitudinal incisions were created on the dorsal skin of Wistar rats. Polyphenols were administered topically during 7 days. The same procedure was performed on Wistar rats that consumed beer or beer supplemented with XN, during 30 days. Beverages consumption was maintained until day 7 after skin incisions. Wounded tissue was then collected for histology and immunohistochemistry analyses. Nitric oxide (NO) release, content in serum were α-N-acetylglucosaminidase (NAG) activity and IL-1 measured. Statistical difference between various groups was evaluated and differences were considered significant whenever p<0.05.

**Results**
XN and IXN treatment led to decreased number of vessels formed and decrease NAG activity, NO content, whereas 8PN presented the opposite effects as well as IL-1 effects. XN also exerted anti-angiogenic and anti-inflammatory properties when administered in beer.

**Discussion/Conclusion**
Interestingly, while 8PN stimulated angiogenesis, XN and IXN manifested anti-angiogenic and anti-inflammatory effects when used topically. The nutritional supplementation of beer with XN confirmed these results. These findings emphasize the distinct effects obtained by compounds with identical chemical structures in angiogenesis and inflammation, providing clues to the development of useful therapeutic agents against inflammation- and angiogenesis-associated pathologies. They also suggest that these polyphenols may affect wound healing process, in what concerns inflammation and angiogenesis, by both topical and gastrointestinal administration.

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Neurosciences

VENCEDOR:

The Acoustic Change Complex and Frequency Discrimination for Subjects with Normal Hearing

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Introduction
The Acoustic Change Complex (ACC) is a component of late auditory evoked potentials (LAEP). It is elicited by change in intensity, periodicity or spectral envelope of the ongoing acoustic stimulus and can be used as an index of ability for perceiving these kinds of acoustic changes, which are important for differentiating speech signals. The purpose of the present study was to compare the parameters of ACC with psychophysical discrimination abilities.

Methods
Twenty normal-hearing adults participated. LAEP were recorded from surface electrodes placed at Fc, Cz, FC1, FC2, F3, F4, C3 and C4 position. The acoustic stimulus lasted 3000 ms and consisted of two parts: the frequency of 1000 Hz presented the first 1500 ms is then increased by an onset of 2, 4, 6, 8 or 10 Hz for another 1500 ms. The psychophysical discrimination for each frequency transition were measured by a 3AFC paradigm.

Results
The ACC occurrence is associated with significant discrimination of frequency transition in the 3AFC. Furthermore some ACC parameters correlated significantly with discrimination results. The P2-Amplitudes increased significantly with increasing stimulus onset. The subjects’ discrimination score of transitions, which did not elicit an ACC, were lower (50-100%) than if an ACC was observed (88-100%). But the difference is not significant.

Discussion/Conclusion
The parameters of ACC were correlated with psychophysical discrimination tests and were further able to objectify them. This can be seen at the smaller variance of 3AFC discrimination scores if the ACC was detectable. If the ACC is detectable, the 3AFC discrimination scores have to be above 88%.

Funding
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