Title:
“Effect of contralateral pure tone stimulation on distortion emissions suggests a frequency specific functioning of the efferent cochlear control”

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Frequency specificity of efferent cochlear control

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Abstract

Contralateral acoustic stimulation (CAS) with white noise and pure tone stimuli was used to assess frequency specificity of efferent olivocochlear control of cochlear mechanics in the gerbil. Changes of the cochlear amplifier can be monitored by distortion product otoacoustic emissions (DPOAEs) which are a byproduct of the nonlinear amplification by the outer hair cells. We used the quadratic DPOAE f2-f1 as ipsilateral probe as it is known to be sensitive to efferent olivocochlear activity. White noise CAS, used to evoke efferent activity, had maximal effects on the DPOAE level for f2-stimulus frequencies of 5-7 kHz. The dominant effect during CAS was a DPOAE level increase of up to 13.5 dB.

The frequency specificity of the olivocochlear system was evaluated by presenting pure tones (0.5-38 kHz) as contralateral stimuli in order to evoke efferent activity. Maximal DPOAE level changes were triggered by CAS frequencies close to the frequency of the DPOAE elicitor tones (tested f2 range: 2.5-15 kHz). The effective CAS frequency range covered 1.4-2.4 octaves and was centered 0.42 octaves below the DPOAE elicitor tone f2. The frequency-specific effect of CAS with pure tones suggests a dedicated central control of mechanical adjustments for peripheral frequency processing.

Introduction

The human auditory system has developed adaptive filtering at different stages of the hearing process, whose control through attentive mechanisms enables us to focus on distinct sound objects composed of specific frequencies (Mondor and Bregman, 1994; Ward and Mori, 1996; Giard et al., 2000; Green and McKeown, 2001). This is relevant, e.g. for speech perception in complex sound environments ("cocktail party effect"; Kuyper, 1972). The inner ear with its efferent feedback innervation could provide a first stage of such adaptive control. However, the frequency specificity of efferent modulation of cochlear function is still a matter of debate.

The cochlear amplifier (Davis, 1983) - the active reinforcement of basilar membrane motion by outer hair cells (OHC) in the mammalian cochlea - provides high inner ear sensitivity and exquisite frequency resolution (for review see e.g. Robles and Ruggero, 2001; Ashmore, 2008). OHCs are directly innervated by the efferent neurons of the medial olivo-cochlear (MOC)
system originating in the brain stem (for review see Guinan and Stankovic, 1996). Activation of the MOC efferents has been shown to modify OHC electromotility and consequently the gain of cochlear amplification. This leads to modulation of a sound induced basilar membrane oscillation and consequently to a modulation of inner hair cell (IHC) receptor potentials and of the auditory nerve fibers’ firing rate (for review see Guinan, 2006).

The MOC is innervated by descending projections from the inferior colliculus (IC) and higher auditory brain areas as well as by ascending projections from the ipsi- and contralateral cochlear nuclei (Warr, 1992). Thus, focused auditory attention (Lukas, 1980; Giard et al., 1994; Puel et al., 1988) as well as an auditory reflex loop (Guinan, 2006) can be the trigger for an activation of the MOC efferents. Anatomical studies show that the afferent innervation of the MOC as well as the efferent MOC projections to the cochleae, are tonotopically organized (Guinan et al., 1984). An efferent nerve fiber terminates approximately at the cochlear site that corresponds to its characteristic frequency, although the frequency tuning of efferent neurons is broader than that of an afferent fiber (Liberman and Brown, 1986).

Distortion product otoacoustic emissions (DPOAEs) are a by-product of the nonlinear transduction process of the OHCs and provide a non-invasive method to probe cochlear activity. They can be used to investigate the impact of efferent activity on the cochlea amplification (for review see Guinan, 2006). Distortion products are evoked by stimulation with two primary tones of different frequencies (f2 and f1; f2>f1) and can be measured simultaneously in the ear canal at distinct frequencies. The two most prominent DPOAEs are the cubic DPOAE with the frequency 2f1-f2 and the quadratic DPOAE (difference tone) at the frequency f2-f1. The quadratic DPOAEs are known to react sensitively to changes in the position of the operating point of cochlear amplification (Frank and Kössl, 1996; Frank and Kössl, 1997) and to efferent manipulation of the inner ear (Kirk and Johnstone, 1993; Chang and Norton, 1997; Abel et al., 2009; Wittekindt et al., 2009).

There are several previous studies using otoacoustic emission (OAE) measurements, which point to a frequency specific control of the cochlea amplification by the olivocochlear efferents (Veuillet et al., 1991; Chéry-Croze et al., 1993; Lilaonitkul and Guinan, 2009). Their results, however, are restricted to a few test frequencies only and in most studies not pure tones, but contralateral narrow band noise stimuli were used to induce MOC activity. To systematically investigate the frequency specificity of efferent modulation, in the present study we focus on the f2-f1 DPOAE and the use of an extended range of primary frequencies as well as contralateral
test frequencies. Further, we used pure tones as contralateral stimuli to narrow down the
frequency range of ipsilateral efferent effects.

Material and Methods

Animals and anesthesia

Nineteen laboratory-bred Mongolian gerbils, *Meriones unguiculatus*, of both sexes were used
for the experiments. Their average age was 6 months, ranging between 5 and 7.5 months. The
bodyweight was between 50 g and 82 g (average 65.5 g). There is no indication of age-related
hearing loss in this age group (Eckrich et al., 2008).

A ketamine/xylazine (Ketavet, Pfizer; Rompun, Bayer) mixture was used for anesthesia. After an
initial subcutaneous injection of approximately 105 mg/kg per bodyweight of ketamine-
hydrochloride and 4.7 mg/kg per bodyweight of xylazine the anesthesia was maintained by a
continuous subcutaneous injection (20-25 % of the initial dose/h), using a syringe pump (Genie,
Kent Scientific). The animal was placed on a heating pad that kept the body temperature
constant at 37.2°C. Its head was fixed with a mouth holder. The animal experiments reported
here comply with the *Principles of Animal Care* (National Institutes of Health, publication 86-23,
revised 1985) and also with German law.

Acoustic system

The acoustic system consisted of three reverse-driven condenser microphones used as
loudspeakers (B&K 4133, ½") and two microphones, one at each side of the animals head (B&K
4190, ½"; B&K 4135, ¼"). The two ipsilateral speakers produced the two primary tones and the
contralateral speaker was used to apply contralateral pure tones and noise stimuli. The
microphones were used for ipsilateral measurement of the DPOAEs and for the bilateral *in situ*
stimulus calibration. The acoustic systems of both sides were connected to custom-built closed
coupler systems whose output tube diameter decreased toward the coupler tip to fit into the
gerbil ear canals. The acoustic system was calibrated *in situ* using white noise (for details of the
acoustic system and calibration procedure see Frank and Kössl, 1996).

All stimuli were generated using custom-written Matlab scripts (version 6.5, The MathWorks)
and two synchronized DA converter boards (DAP 840 and DAP 4200; Microstar Laboratories).
The stimuli were attenuated (PA5, Tucker Davies Technologies) and amplified (custom-made amplifier) before driving the loudspeakers. The microphone signal was amplified (B&K Type 2669 preamplifier and Type 2610 measuring amplifier), sampled at 100 kHz and stored for further analysis.

**Measurements**

Two pure tone stimuli with the frequencies $f_1$ and $f_2$ ($f_2>f_1$) were presented at equal levels ($L_1=L_2$) for 2620 ms (50 ms rise/fall) in order to evoke DPOAEs in the ipsilateral ear channel. While the $f_2$ frequency was kept constant at 2.5, 3, 5, 7, 10 or 15 kHz, the $f_1$ frequency was chosen for each animal individually such that high $f_2$-$f_1$ levels could be obtained. The preferred $f_2/f_1$ ratio was 1.3. The contralateral stimulus (50 ms rise/fall) was applied in a period between 1000 and 1800 ms after onset of the ipsilateral two-tone stimulus. Each recording was repeated up to 4 times depending on the background noise level and recordings with visually identified artifacts were rejected. After time averaging the frequency spectrum was calculated using fast Fourier transformation (FFT, hanning window).

To assess general susceptibility of DPOAE to contralateral stimulation, in a first experimental step, DPOAE growth functions were recorded by increasing the primary tone level stepwise ($\geq 12$ dB SPL up to 63 dB SPL, step size 3 dB) and using white noise (50 dB SPL) as contralateral stimulus.

In a second experimental step, pure tones with varying frequencies were used as contralateral stimuli. The contralateral pure tones had a level of 50 dB SPL and were either presented in a single series of increasing frequency (26 steps, 1/4 octave steps, starting at 0.5 kHz) or in three consecutive frequency series that allowed to rule out DPOAE magnitude shifts during the course of the experiment. The primary tone levels for these measurements were chosen within a range of 28 to 57 dB SPL, at levels where prior contralateral white noise exposure had produced largest changes in the $f_2$-$f_1$ DPOAE growth functions. We avoided primary levels associated with pronounced notches in the growth function. In such cases, a primary tone level slightly lower than that producing the notch was used.

**Data analysis**
DPOAE magnitudes were extracted by FFT in the three periods before (ms 500-1000), during (ms 1300-1800) and after (ms 2070-2570) contralateral acoustic stimulation (see Fig. 1A). These time windows were chosen to exclude DPOAE onset adaptation behavior and on- and offset of the CAS-induced effects.

The background noise level was determined by averaging the amplitude of six data points around the f2-f1 DPOAE frequency in the spectrum. The noise level threshold was defined for each test series (growth function or frequency run) at two standard deviations above the highest mean noise level of the periods before, during or after CAS (see Fig. 1). Only DPOAE recordings exceeding the respective noise threshold were taken into further analysis.

To assess the size of the CAS induced DPOAE level shift per growth function the absolute DPOAE level shift during CAS relative to before CAS was calculated and averaged for primary levels between 27 and 57 dB SPL. Absolute values were taken, because the DPOAE level shift during CAS could be either positive or negative and with this quantification the general effect strength of CAS with noise on the DPOAE level could be analyzed.

To illustrate the direction of DPOAE level change and the variance in the effect size, the maximum DPOAE level change during CAS (relative to the level before CAS) was calculated for each growth function among primary level between 27 and 57 dB SPL. Further, to examine possible offset effects the DPOAE level change after CAS (relative to before CAS) was calculated for the same measurement.

The DPOAE level shift induced by contralateral pure tone stimulation was calculated by taking the difference between the DPOAE level during CAS relative to the mean DPOAE level before and after CAS. We determined the effect direction (either level enhancement or reduction) of all measurements within a CAS frequency range -2 to +1 octaves around the ipsilateral f2 frequency. Only test series with more than 70% of the measurements showing an effect in the same direction were taken into further analysis (this accounts to about 85% of all measured series). In the following, test series with a DOPAE level enhancement were averaged separately from those with a DPOAE level decrease. Furthermore, the bandwidth of the contralateral frequencies inducing a half maximal f2-f1 DPOAE level shift was calculated and its center frequency (in octaves) was defined as the most effective CAS frequency (see Fig 3B). We did not use the single CAS frequency inducing the maximal level shift since it was often more vulnerable to artifacts and the center frequency gave a more robust estimate of the CAS frequency being most effective.
A linear regression analysis of the most effective CAS frequencies was calculated (jmp, Six Sigma).

Results

Basic effects of contralateral stimulation with white noise on the DPOAE level

In most cases, contralateral acoustic stimulation with white noise induced a clear change of the f2-f1 DPOAE level evoked by ipsilateral two tone stimulation with moderate primary levels (for a representative example see Fig. 1B-D and E). The CAS induced effect predominantly consisted of a DPOAE level increase. After CAS offset, an off-effect with a DPAOE level shift into the opposite direction (compared to the shift during CAS) beyond the initial pre-CAS baseline level, was often observed. The simultaneously measured cubic 2f1-f2 DPOAE level was not or only slightly affected by the CAS (s. Fig. 1B-D and F). In the example shown in Fig. 1C, the f2-f1 DPOAE level increased during CAS by about 8.1 dB, whereas the 2f1-f2 DPAOE level only changed by about 0.2 dB.

To assess the relation between the primary tone level and the CAS-induced DPOAE level shift, DPOAE growth functions were measured. For the example shown in Fig. 1E, a CAS induced f2-f1 DPOAE level enhancement was observed for primary tone levels up to 50 dB SPL whereas no or only minimal level shifts of the 2f1-f2 DPOAE could be measured (Fig. 1F).

Impact of white noise CAS on f2-f1 DPOAE for different primary tone frequencies

Representative examples of f2-f1 DPOAE growth functions for different f2-primary tone frequencies with white noise CAS (50 dB SPL) are shown in Fig. 2A-D. As the course of the curves was variable, the growth functions were not averaged. In general the DPOAE level increased with increasing primary tone levels. However, the course of the DPAOE growth function often was nonlinear with either a local reduction of the slope (a plateau; see Fig. 2A and B) or one or two notches of the DPOAE level at distinct primary tone levels (see Fig. 2C and D). The shape of the f2-f1 DPOAE growth functions depended on the primary tone frequency and there was a trend towards a more nonlinear course and more pronounced notches for stimulation with higher frequencies. CAS induced changes of the f2-f1 DPOAE were assessed using low to medium primary levels of up to about 57 dB SPL. In most cases a level
increase (Fig. 2A, B and D) was observed. However also level decreases were possible (Fig. 2C). Generally, the direction of the effect (increase or decrease) was consistent per growth function over the tested primary level range. The relative position of the DPOAE level notch could shift during CAS to higher or lower primary tone levels (e.g. Fig 2C).

The CAS induced f2-f1 DPOAE level shift was evaluated for the different f2 frequencies by calculating the average absolute f2-f1 DPOAE level shift for the measurements obtained with primary tone levels from 27 to 57 dB SPL (Fig. 2E). This implies that both positive and negative effects were taken into account.

For f2 = 2.5 and 3 kHz a moderate mean DPOAE level change of 1.3 dB could be observed (Fig. 2E). Growth functions for f2 = 5 kHz in general showed a larger average DPOAE level shift of 2.1 dB during CAS. The CAS induced DPOAE level change reached a maximal value of 2.2 dB for f2 = 7 kHz. For higher primary frequencies, it became smaller again (1.3 dB for f2 = 10 kHz, 1.5 dB for f2 = 15 kHz; Fig. 2E). We also checked if the mean DPOAE level shift was substantially different when only level enhancements were taken into the analysis. This was not the case.

To demonstrate the distribution of positive and negative DPOAE level shifts during CAS, the maximum DPOAE level change of each growth function (within the primary tone level range 27 to 57 dB SPL) is plotted in figure 2F (circles). Positive and negative level shifts reached maximum values of 13.5 and -10.9 dB, respectively. The main direction of level changes during CAS was positive (88 % of the cases). Corresponding level shifts after CAS offset (relative to the DPOAE level before CAS) are indicated by the squares in Figure 2 F (lines connect related data points). In 44% of the cases we found a CAS offset effect where the DPOAE level was shifted into the opposite direction (compared to the shift during CAS) beyond the initial pre-CAS baseline level. The offset effects can amount to 4.8 dB in positive and -7.7 dB in negative direction (Fig 2F).

**Frequency specific effect of pure tone CAS on the f2-f1 DPOAE level**

To investigate a frequency specific impact of CAS on the f2-f1 DPOAE level, pure tones with different frequencies (range 0.5 and 38 kHz) were applied as contralateral stimuli.

Maximal ipsilateral f2-f1 DPOAE level changes of 1-3 dB were induced by contralateral pure tones which were in the range of the ipsilateral primary tone frequencies (see Fig. 3 and 4).
Towards higher and lower contralateral frequencies, the CAS induced DPOAE level shift decreased and was absent for the most distal frequencies (Fig. 3 and 4). The observed DPOAE level shift during CAS was predominantly positive (in 86% of the cases) and a negative DPOAE level shift was only found in cases where $f_2 > 7$ kHz (see Fig. 4). The strength of the DPOAE level shift differed for the different $f_2$ frequencies being largest for $f_2 = 7$ kHz (Fig. 4) and furthermore offset effects after CAS were often observed (see example in Fig. 3A).

The bandwidth of the contralateral frequency range that induced a half maximal $f_2$-$f_1$ DPOAE level shift varied between 2.36 and 1.41 octaves and was not significantly different for the tested $f_2$-frequencies. The most effective CAS frequencies correlated with the $f_2$-primary tone frequencies (Fig. 5, $n = 66$; $R^2 = 0.58; p < 0.0001^*$), but were slightly lower in frequency. On average the most effective CAS frequency was 0.42 octaves below the corresponding $f_2$ frequency (range: +0.9 to -1.51 octaves).

**Discussion**

This work gives evidence for a frequency specific regulation of the cochlear amplifier, mediated by medial olivocochlear efferents which were activated by contralateral acoustic stimulation. The study focuses on the effect of CAS with white noise and pure tones on the $f_2$-$f_1$ DPOAE, which is known to be sensitive to changes in the operating state of cochlear amplification (Frank and Kössl, 1996; Bian, 2004) and to react strongly to contralateral stimulation (Kirk and Johnstone, 1993; Kujawa et al., 1993; Chang and Norton, 1997; Wittekindt et al., 2009). Therefore the stimulation parameters were optimized to produce maximal $f_2$-$f_1$ level. As seen in previous studies, the $2f_1$-$f_2$ level was much less affected by CAS (e.g. gerbil: Abel et al., 2009; human: Wittekindt et al., 2009).

We cannot fully exclude the possibility that the middle ear muscle reflex contributes to the effects. The used CAS level of 50 dB SPL, however, was below that demonstrated to activate middle ear muscles in rodents (Pilz et al., 1997) and the frequency specificity of the DPOAE level changes argues against middle ear muscle mechanisms.

The results of this study mainly concern fast MOC effects. As shown by Cooper and Guinan (2003), slow effects change the cochlear amplification after a continuous stimulation of the MOC efferents of 10s of seconds while fast effects have a time constant of about 100ms. In the present study we always calculated the difference between the DPOAE level during CAS of
800 ms duration and the DPOAE level just before the stimulation. Therefore the observed level
shifts reflect changes of cochlear amplification on a fast time scale. Further, in a previous study
(Abel et al., 2009) we also measured the time constant of the CAS induced level shift using a
comparable paradigm. We found time constants of 56 ms and 52 ms for the onset and offset of
the CAS induced level shift, respectively. This again argues for fast efferent effects.

Effect of white noise CAS on the f2-f1 DPOAE

The present study investigates the effect of short term (800 ms) white noise CAS on f2-f1
dPOAE growth functions in the gerbil for a wide range of ipsilateral stimulation frequencies. This
extends previous studies using only a restricted range of primary frequencies (gerbil: Abel et al.,
2009; guinea pig: Mountain, 1980; Kirk and Johnstone, 1993; chinchilla: Siegel and Kim, 1982;
human: Wittekindt et al., 2009). Maximum f2-f1 level changes (predominantly level increases) of
about 13.5 dB and average changes close to 2 dB were found for f2 frequencies of 5 and 7 kHz.
Whereas in the Gerbil CAS induced f2-f1 level shifts are predominantly positive, as shown by
the present study and a study by Abel et al. (2009), in humans and the guinea pig, a f2-f1 level
decrease is found during white noise CAS (Chang and Norton, 1997; Wittekindt et al., 2009).
Since the level of the f2-f1 DPOAE depends on the location of the cochlear amplifier operating
point (Frank and Kössl, 1996; Frank and Kössl, 1997), in the gerbil efferent activation would
shift the OP mainly toward asymmetry, whereas in the guinea pig and human, the dominant shift
would be towards symmetry. This however does not mean that the efferent mechanisms differ in
these species. A possible explanation is that the resting position of the OP is either on the
negative or positive side of the inflection point of the transfer function in these species. Thereby
a shift of the OP in the same direction would result in a shift towards or away from the symmetry
and consequently in a f2-f1 level decrease or increase.

As it is described for 2f1-f2 DPOAE growth functions in the gerbil (e.g. Brown, 1987; Mills,
2002; Frank and Kössl, 1996), also the course of f2-f1 growth functions in the present study was
characterized by plateaus and notches. During CAS the f2-f1 level shift in the notch regions was
usually much stronger than in the monotonic parts of the growth function. Interestingly, CAS
could shift the relative position of the notch towards higher or lower primary tone levels.
Assuming that the notch is produced by a transition of the contribution of different DPOAE
generation mechanisms (Mills and Rubel, 1994) the observed shifts could indicate that the
efferent modulation mainly affects one of the generation mechanisms. Alternatively, the
nonlinear DPOAE growth including the notch may be produced by a single nonlinearity (Lukashkin and Russell, 2002, Lukashkin and Russell, 2005, Kössl and Coro, 2006). Accordingly, a shift of the notch would be due to a shift in the OP of the cochlear nonlinearity and the direction of the change in notch position could predict the direction of the operating point shift (Lukashkin and Russell, 2002). Lukashkin and Russell (2002) could partially prevent such shifts in 2f1-f2 growth functions by administration of strychnine which blocks efferent synaptic activity.

However, it has to be emphasized, that changes in the f2-f1 DPOAE level also could be produced by efferent effects on general gain of cochlear amplification, as it has been indicated for basilar membrane mechanics (Murugasu and Russell, 1996) and auditory nerve activity (Gifford and Guinan, 1983) in previous studies (review: Russell and Lukashkin, 2007).

We observed offset effects on the f2-f1 DPOAE level after CAS (see also Abel et al., 2009). In literature, pronounced offset effects regarding OAE level and hearing sensitivity are usually found after loud ipsilateral low frequency stimulation ("Cochlear bounce": Hirsh and Ward, 1952, Kemp, 1986, Kirk and Patuzzi, 1997, Kirk et al., 1997; Kevanishvili et al., 2006). This phenomenon commonly was interpreted as operating point shift and changes in permeability of the cochlear organ, standing currents through hair cells, and osmotic imbalance were suspected as source of such an operating point shift (Kirk and Patuzzi, 1997, Kirk et al., 1997). Offset effects are also reported after moderate ipsilateral sound stimulation in the cochlear summating potential (e.g. Harvey and Steel, 1992) or hair cell dc receptor potentials (e.g. Russell and Sellick, 1978, Russell and Kössl, 1992) and are also discussed in terms of an OP shift (see Sirjani et al., 2004).

The level of the simultaneously measured 2f1-f2 DPOAE was only slightly influenced by CAS. This matches previous results of recordings from the gerbil (Abel et al., 2009), the guinea pig (Kirk and Johnstone, 1993; Chang and Norton, 1997) and humans (Wittekindt et al., 2009). The different reactions of the cubic and the quadratic DPOAEs could be due to the fact that the f2-f1 DPOAE reacts more sensitively to a shift of the OHC operating point when it is around its point of symmetry, than the 2f1-f2 DPOAE (Frank and Kössl, 1996).

Effect of pure tone CAS on the f2-f1 DPOAE
The frequency dependent behavior of the f2-f1 DPOAE level during CAS with pure tones that was found in this study confirms exemplary data from the gerbil (Abel et al., 2009) and is a possible functional correlate of frequency specific MOC projections into the cochlea (Liberman and Brown, 1986). The data are consistent with frequency specific effects of narrowband noise CAS that were found for frequencies around 1 to 2 kHz (click-evoked OAEs: Veuillet et al., 1991; 2f1-f2 DPOAE: Chéry-Croze et al., 1993) and with the results of a study by Lilaonitkul and Guinan (2009). In the latter study, the frequency selectivity of the human cochlea was assessed by measuring stimulus frequency OAE (SFOAE) elicited by a probe tone at a frequency of 1 kHz. In order to elicit MOC activity, the authors stimulated for 2.5 s with a 60 dB SPL tone or a narrow band noise and found a frequency specific effect that is similar to our results. CAS-frequencies near the SFOAE-elicitor frequency, that is 1 kHz, caused larger MOC effects than CAS-frequencies which were more distant from the SFOAE-elicitor frequency. There was a skew to lower frequencies, as maximal MOC effects were produced by tones 1.5 to 0.5 octaves below the SFOAE-elicitor frequency. A similar relationship is evident in our data, as the most effective CAS frequency was 0.42 octaves below the f2 frequency.

In our study, the bandwidth of CAS frequencies which was sufficient to induce a half-maximal DPOAE level shift ranged between 1.41 and 2.36 octaves for the different f2-frequencies. Studies in the guinea pig indicate that up to 61 OHCs are innervated by one efferent fiber, which would correspond to a cochlear region which covers less than one octave (Brown, 1989). However, as a consequence of the tuning bandwidth of auditory afferents that innervate the efferent neurons in the medial olivocochlear complex, a corresponding widening of the bandwidth of the frequency specific MOC effect would follow.

It can be concluded that MOC efferents allow an adjustment or control of the operating state and/or gain of the cochlear amplifier in a frequency-specific way over a wide range of frequencies covering a substantial part of the hearing range in the gerbil. From the f2-f1 DPOAE data alone it is difficult to assess the possible types of adjustment in terms of e.g. changes in auditory sensitivity or tuning sharpness. However, in our stimulation paradigm, with a dominant increase of the f2-f1 DPOAE level, the operating point of the auditory nonlinearity should be biased towards asymmetry which may decrease auditory sensitivity and gain. The functional tonotopic characteristics of efferent action, as indicated by the present and other studies, provide a lever to modulate inner ear input to the central auditory system in dependence of frequency content of input signals, as it should be of advantage during complex signal processing and selective auditory attention.
Acknowledgments

This study was supported by the DFG, KO987/11-1.

References


**Figure legends**

**Fig.1** General effect of white noise CAS on f2-f1 and 2f1-f2 DPOAE level. A. Stimulation paradigm (2.62 s stimulation with the primary tones f1 and f2; 800 ms of CAS stimulation). B-D. Frequency spectra of one single recording (f1 = 5.38 kHz, f2 = 7 kHz; L1, L2 = 42 dB SPL, CAS = 50 dB SPL) for the stimulation periods before, during and after white noise CAS (indicated by the windows in A). Marked peaks indicate the two primary tones (f1, f2), the f2-f1 DPOAE (marked by a circle) and the 2f1-f2 DPOAE (marked by an asterisk). Dotted horizontal lines indicate the f2-f1 and 2f1-f2 DPOAE levels before CAS. Straight gray line gives the noise level threshold for f2-f1 (see material and methods). E and F. Corresponding growth functions of the f2-f1 DPOAE (E) and the 2f1-f2 DPOAE (F) level before (solid line), during (dashed line) and after CAS (grey line). Dotted gray lines indicate the background noise level for the respective analysis windows. The horizontal black dotted line gives the noise level threshold (see material and methods).

**Fig.2** Effect of CAS with noise on DPOAE growth functions. Characteristic examples of f2-f1 DPOAE growth functions (A-D) at different f2 frequencies before (black lines), during (dashed lines), and after (grey lines) CAS with 50 dB SPL white noise. Thin vertical lines give the primary level range (27-57 dB SPL) used for the analysis shown in Fig 2E. Dotted lines indicate the noise level threshold (see material and methods for details). E. Mean of the absolute f2-f1 DPOAE level shifts during CAS (relative to the level before CAS and averaged for the L2 level range 27-57 dB SPL; n = 9 for 2.5 kHz, 13 for 3 kHz, 15 for 5 kHz, 16 for 7 kHz, 7 for 10 kHz and 4 for 15 kHz). Error bars indicate the standard error of the mean. F. Circles give the largest CAS induced DPOAE level change per growth function (during relative to before CAS). Squares indicate the respective DPOAE level shift after CAS offset (relative to before CAS). The two values of each recording are connected with a line.

**Fig.3** Influence of contralateral pure-tone stimulation on the f2-f1 DPOAE level. A. f2-f1 DPOAE level before (black curve), during (dashed curve) and after (grey curve) contralateral pure tone stimulation with frequencies between 0.5 kHz and 38 kHz (f2 = 3 kHz; f1 =2.14 kHz; L1, L2 = 40 dB SPL; CAS level = 50 dB SPL). Vertical line marks the f2 frequency at 3 kHz. B. Difference curve from the same recording showing the level shift of the f2-f1 DPOAE during CAS relative to the mean DPOAE level before and after CAS. The dotted line gives the bandwidth of the curve.
at the half maximal level shift. The cross marks the center (in octaves) of the bandwidth (at 2.3 kHz) which was defined as the most effective CAS frequency.

Fig.4 Effect of contralateral pure tone stimulation on the f2-f1 DPOAE level for different primary tone frequencies. Average positive (A-F) and negative (G,H) shifts of f2-f1 DPOAE levels during CAS with pure tones (CAS level = 50 dB SPL). For f2 = 7 and 10 kHz both the positive (D,E) and the negative shifts (G,H) are shown. Vertical grey line gives the f2-primary tone frequency. Error bars indicate the SEM.

Fig.5 The most effective contralateral pure tone frequency (to induce a f2-f1 level shift) correlates with the ipsilateral primary tone frequency f2. Grey line gives the linear regression of the data (n = 66; R² = 0.58; p < 0.0001*). Dotted line gives a slope of 1.
A

before CAS after CAS

DPOAE level [dB SPL]

B

f1 f2

2f1-f2

f2-f1

ipsi-

lateral

contra-

lateral

C

D

Frequency [kHz]

Ear canal level [dB SPL]

E

f2-f1 DPOAE

F

2f1-f2 DPOAE

DPOAE level [dB SPL]

L1=L2 primary tone levels [dB SPL]
f2 = 3 kHz
f1 = 2.3 kHz

f2 = 5 kHz
f1 = 3.8 kHz

f2 = 7 kHz
f1 = 6.14 kHz

f2 = 10 kHz
f1 = 7.7 kHz

A

B

C

D

E

F

L1=L2 primary tone level [dB SPL]
A. f2-f1 DPOAE-level [dB SPL]

B. f2-f1 DPOAE level shift [dB]

CAS-frequency [kHz]

Before CAS
During CAS
After CAS

Most effective CAS frequency
A: $f_2 = 2.5$, $n = 6$

B: $f_2 = 3$, $n = 14$

C: $f_2 = 5$, $n = 14$

D: $f_2 = 7$, $n = 9$

E: $f_2 = 10$, $n = 8$

F: $f_2 = 15$, $n = 7$

G: $f_2 = 7$, $n = 4$

H: $f_2 = 10$, $n = 3$
most effective CAS frequency [kHz] vs. f2-frequency [kHz]