Contralateral Acoustic Stimulation Modulates Low-Frequency Biasing of DPOAE: Efferent Influence on Cochlear Amplifier Operating State?

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Abel C, Wittekindt A, Kössl M. Contralateral acoustic stimulation modulates low-frequency biasing of DPOAE: efferent influence on cochlear amplifier operating state? J Neurophysiol 101: 2362–2371, 2009. First published March 11, 2009; doi:10.1152/jn.00026.2009. The mammalian efferent medial olivocochlear system modulates active amplification of low-level sounds in the cochlea. Changes of the cochlear amplifier can be monitored by distortion product otoacoustic emissions (DPOAEs). The quadratic distortion product f2–f1 is known to be sensitive to changes in the operating point of the amplifier transfer function. We investigated the effect of contralateral acoustic stimulation (CAS), known to elicit efferent activity, on DPOAEs in the gerbil. During CAS, a significant increase of the f2–f1 level occurred already at low contralateral noise levels (20 dB SPL), whereas 2f1–f2 was much less affected. The effect strength depended on the CAS level and as shown in experiments with pure tones on the frequency of the contralateral stimulus. In a second approach, we biased the position of the cochlear partition and thus the cochlear amplifier operating point periodically by a ipsilateral low-frequency tone, which resulted in a phase-related amplitude modulation of f2–f1. This modulation pattern was changed considerably during contralateral noise stimulation, in dependence on the noise level. The experimental results were in good agreement with a simple model of distortion product generation and suggest that the olivocochlear efferents might change the operating state of cochlear amplification.

INTRODUCTION

Outer hair cells (OHCs) are one of the key elements of nonlinear cochlear amplification, which is responsible for high inner ear sensitivity and exquisite frequency resolution (for review see Ashmore 2008; Dallos et al. 2006; Robles and Ruggero 2001). They are densely innervated by efferent fibers that originate in the brain stem and can modulate the electromotive response characteristics of OHC and cochlear mechanics (reviews: Guinan 1996; Russell and Lukashkin 2008). Details of the physiological mechanisms of the medial olivocochlear (MOC) efferent system, its influence on the cochlear mechanics, and its biological function are still under discussion. Activation of the olivocochlear bundle has a substantial, predominantly suppressive effect on basilar membrane motion (e.g., Cooper and Guinan 2003; Murugus and Russell 1996; Russell and Murugus 1997) and auditory nerve activity (e.g., Guinan and Gifford 1988a; Guinan et al. 2005; Wiederhold and Kiang 1970) in response to low-level tones. The influence of efferent activity on the cochlear amplification can be investigated by recording otoacoustic emissions (for review see Guinan 2006) that are generated as a by-product of amplification of low-level sound by outer hair cells (Kemp 2002; Probst et al. 1991).

In the present study, distortion product otoacoustic emissions (DPOAEs), evoked by simultaneously stimulating the ear with two pure-tone stimuli (primary tones) of different frequencies f1 and f2, were used to assess cochlear response characteristics. The efferent MOC pathway was activated by contralateral acoustic stimulation (CAS) with white noise. Experiments including sectioning of the olivocochlear bundle (Puel and Rebillard 1990) and suppression of the CAS-induced effects by specific antagonists of the medial olivocochlear neurotransmitter acetylcholine (Kujawa et al. 1993) could attribute the effects of CAS on DPOAEs to efferent nerve fibers.

Analysis of the different types of DPOAEs, cubic distortion tones (CDTs, e.g., 2f1–f2) and quadratic distortion tones (QDTs; e.g., f2–f1), can describe changes in the gain and the operating state of the cochlear amplifier. In experiments applying acoustical and electrical biasing of the cochlear partition to induce periodical shifts of the operating point (OP) of the cochlear amplifier transfer function, it could be shown that the QDT f2–f1 is a very sensitive indicator for changes in the OP of the transfer function if the OP is situated near the point of inflection (Bian 2004; Frank and Kössl 1996, 1997; Lukashkin and Russell 2005). The generation of cochlear two-tone distortions depends on the transfer characteristic of auditory transduction and can be modeled by using a nonlinear transfer function, such as a second-order Boltzmann function (for further details see METHODS), similar to those that are used to describe mechanoelectrical transduction (e.g., Kros et al. 1992). Figure 1 illustrates the dependence between the OP of a putative cochlear amplifier transfer function and the resulting magnitude of the 2f1–f2 and f2–f1 distortion products (Fig. 1, A–D). The simulation further shows periodical changes in distortion product (DP) levels during additional stimulation with a low-frequency tone that introduces periodical shifts of the operating point on the transfer function (Fig. 1, E and F). The simulation illustrates that small changes in the OP induce large changes in the f2–f1 amplitude, whereas the 2f1–f2 amplitude is much less affected. This implies that it is useful to focus on measurement of the f2–f1 distortion product for describing potential efferent effects on the operating state of cochlear amplification. In those studies on efferent-induced effects on DPOAEs that also analyze alterations of the QDT f2–f1, the efferent influence on f2–f1 was often more pronounced than that on 2f1–f2 (Chang and Norton 1997; Kirk and Johnstone 1993; Wittekindt et al. 2009). This leads to the hypothesis that efferent activity could indeed change the operating state of the cochlear amplifier. To test whether changes in the operating point of the
cochlear amplifier as they are induced by low-frequency biasing are comparable to efferent effects induced by CAS, in the present study both types of acoustic stimulation were used simultaneously and their impacts on DPOAEs were compared. The results are interpreted by means of the simple model using a Boltzmann function (see Fig. 1 and METHODS) to simulate the consequences of OP shifts on distortions.

**METHODS**

Fourteen laboratory-bred Mongolian gerbils of both sexes (40–70 g bodyweight) were used in the present study. The age of the animals was between 4 and 9 months, in a range where age-related hearing loss occurs in the level of 2f1–f2 DPOAE does not yet occur (Eckrich et al. 2008). The animals were anesthetized with an initial injection of ketamine (Ketavet, 105 mg/kg bodyweight) and xylazine (Eckrich et al. 2008). The animals were anesthetized with an initial injection of ketamine (Ketavet, 105 mg/kg bodyweight) and xylazine (Eckrich et al. 2008).

The acoustic system consisted of two reverse-driven condenser microphones (B&K 4133, ½-in.) serving as loudspeakers to apply the primary tones, one headphone loudspeaker (DT 880, Beyerdynamic) for presentation of a low-frequency biasing tone, and a microphone (B&K 4190, ½-in.) for measuring the acoustic signal at the tympanum. The two speakers were connected to a custom-built closed coupler system whose output tube diameter decreased toward the coupler tip to fit into the gerbil ear canal. The microphone was connected to a similar tube and both tubes were glued together so that their tip openings were aligned. The acoustic system was calibrated in situ using white noise (for details of the acoustic system and calibration procedure see Frank and Kössl 1996). For contralateral acoustic stimulation a single reverse-driven microphone (B&K 4133, ½-in.), calibrated before the experiment, was connected to a small plastic tube placed into the opposite meatus.

All stimuli were generated using custom-written Matlab scripts (version 6.5, The MathWorks) and two synchronized DA converter boards (DAP 840, Microstar Laboratories), attenuated (PAS, Tucker Davies Technologies) and amplified (custom-made amplifier). The microphone signal was amplified (B&K Type 2669 preamplifier and Type 2610 measuring amplifier), sampled at 100 kHz, and stored for further analysis. A two-tone stimulus (2.62-s duration, 50-ms cosine rise/fall flank) was used to evoke DPOAEs. In all experiments, primary tones were adjusted to f1 = 5.47 kHz and f2 = 7 kHz (f2/f1 = 1.28) at equal levels L1 = L2 (9–60 dB SPL, step size 3 dB).
such that large f2–f1 and 2f1–f2 DPOAE levels could be measured. In the experiments with low-frequency biasing, a 5-Hz bias tone was additionally presented to the ipsilateral ear at various sound pressure levels (89–109 dB SPL, step size 2 dB). Contralateral acoustic stimuli were applied for 800 ms with a delay of 1,000 ms to the onset of the ipsilateral two-tone stimulus. The contralateral stimulus was white noise with various levels between 10 and 70 dB SPL (step size 10 dB).

In several additional experiments, the CAS stimulus consisted of pure tones, adjusted to levels of 50–70 dB SPL (step size 10 dB) and frequencies between 0.5 and 19,027 kHz, increased stepwise by 0.25 octaves. In one of these measurements, the primary-tone frequencies differed (f1 = 3.9 kHz, f2 = 5 kHz, f2/f1 = 1.28).

The acoustic signal from the ear channel was time averaged two to five times and DPOAE magnitudes were extracted using a moving-window method (Hanning window, 1,667 data points) followed by fast Fourier transformation (FFT) analysis. In initial experiments five averages were used; in the course of the study, we increased the number of measurements during an experiment and therefore reduced the number of averages. The time window covered a range of 16.67 ms (according to 30° of the bias tone) and was overlapping by 20% such that FFTs were obtained every 13.33 ms of the 2.62-s stimulation period. The background noise level was determined by averaging the amplitude of six data points around the f2–f1 frequency in the spectrum. To describe the time course of the CAS-induced effects on DPOAEs in the experiments without biasing, the time constant τ was calculated by fitting an exponential function to the data recorded at a CAS level of 60 dB SPL in the time period during (1,000–1,800 ms) and after (1,800–2,620 ms) CAS.

DPOAE amplitudes were additionally extracted by FFTs calculated for three longer timeframes (see Fig. 2)—before CAS (500–1,000 ms), during CAS (1,300–1,800 ms), and after CAS (2,120–2,620 ms)—and taken for further statistical analysis (JMP software, version 7.0; SAS Institute). These time windows were chosen to exclude DPOAE onset adaptation behavior and on- and offset of the CAS-induced effects.

In biasing experiments, the DPOAE amplitude was averaged across and plotted against the corresponding phases of the bias tone for periods before, during, and after CAS separately.

In addition to the experimental approach, the DP response during low-frequency biasing was simulated using a simple model based on a Boltzmann function, similar to the models used by Frank and Kössl (1996) and Lukashkin and Russell (1999)

\[
y = \frac{1}{1 + e^{0.15 - x - y / 0.15}} \left(1 + e^{0.15 - x - y / 0.15}\right)
\]

The output of the nonlinear function (Eq. 1) for a double-sinusoidal input (f1 = 5.47 kHz, 2 dB re 0.1; f2 = 7 kHz, 2 dB re 0.1) was calculated and magnitudes of the cubic and quadratic DPs were extracted with FFT. The nonlinearity is considered in the formula where x is the input (f1 and f2); \(a_2, a_1, x_2, x_1\) are constants (\(x_1 = x_2 = -0.06; a_1 = 3a_2 = 12.8\); and \(b\) is an additional 5-Hz (~10 dB re 0.1) sinusoidal input, at the same frequency as the low-frequency bias tones in the experiments. Variable \(s\) is used to set the resting position of the OP of the nonlinear function. The simulated DP levels (see Fig. 1) for different bias tone amplitudes (\(b\)) and OP resting positions (\(s\)) were qualitatively compared with the experimental data.

The animal experiments reported here comply with the Principles of Animal Care (National Institutes of Health, publication 86-23, revised 1985) and also with the Declaration of Helsinki.

RESULTS

Basic effects of contralateral noise stimulation on f2–f1 and 2f1–f2 DPOAE levels

The DPOAEs were clearly affected by CAS during the continuous two-tone stimulation paradigm that included inter-

mittent contralateral white noise stimulation. During the period of CAS, a distinct level shift of the quadratic f2–f1 distortion was observable, which was a level increase in the majority of experiments (for a representative example, see Fig. 2). The strength of this f2–f1 level enhancement depended on the CAS level. Under the same stimulation condition, the simultaneously measured cubic 2f1–f2 DPOAE was not or only slightly affected. Effects of increasing CAS levels on DPOAEs were determined in 14 animals. In 12 animals, the f2–f1 amplitude increased during CAS; in 2 animals it decreased (CAS level range tested 10–70 dB SPL). In these experiments the primary-tone levels were chosen arbitrarily for each animal within a range of 30–55 dB SPL.

To systematically investigate the dependence of the effect strength and direction on the primary tone level, DPOAE...
growth functions were measured in 10 gerbils by increasing the primary-tone levels systematically. Two examples of such DPOAE growth functions, calculated from FFT analysis in timeframes before and during CAS (50 dB SPL), are shown in Fig. 3. In all measurements, the 2f1–f2 level growth functions did not show any conspicuous notches up to the highest stimulus levels (60 dB SPL). CAS did not affect (or only slightly affected) the 2f1–f2 level within the primary stimulus range tested. The growth function of the f2–f1 distortion was highly nonlinear with a small slope at low primary-tone levels and typically with a distinct f2–f1 level notch at stimulus levels around 45–50 dB SPL. Two different subtypes of CAS-induced effects on the f2–f1 growth functions could be distinguished: In the majority of animals (n = 7), an f2–f1 level enhancement at low primary levels (see example in Fig. 3A; see also Fig. 2) was observed and only in the notch region, opposite effects (level suppression) could occur. In a minority of cases (n = 2), CAS induced a clear decrease of the f2–f1 level at low primary levels and an enhancement occurred only in the notch region (Fig. 3B). One animal could not be classified into one of the two groups. For high primary-tone levels a CAS-induced effect could no longer be observed in any animal.

To quantify the dependence of DPOAE level shift on the strength of contralateral stimulation, in 12 gerbils that showed increasing f2–f1 levels (see earlier text), DPOAE data from measurements with constant primary-tone levels (30–55 dB SPL, individually chosen in each animal; see earlier text) and increasing CAS levels (usually 10–70 dB SPL, increased stepwise by 10 dB) were averaged (Fig. 4). The data show a clear dependence of the f2–f1 level increase (Fig. 4A) on the CAS level. A statistically significant influence of CAS on f2–f1 could be verified from CAS levels as low as 20 dB SPL (Wilcoxon signed-rank test, difference from zero). At high CAS levels, f2–f1 was increased by up to 5.1 dB on average (individual maximal shift: +10.4 dB). Furthermore, small 2f1–f2 level decreases were found at high CAS levels (average maximal shift: −0.24 dB; individual maximal shift: −0.8 dB; shifts were significant at 40, 60, and 70 dB SPL CAS level). After CAS offset, the f2–f1 level dropped to values that were significantly below the levels obtained before CAS stimulation (Fig. 4B). This phenomenon is also evident in the exemplary data shown in Fig. 2.

The time course of both the f2–f1 DPOAE level enhancement during CAS and the decline after CAS was characterized by the time constants τ. The mean calculated time constant τ was 52.0 ms (SD 20.4 ms, n = 10) for the effect onset and 56.7 ms (SD 30.0 ms, n = 10) for the effect offset.

**Frequency specificity of the CAS effect**

In four gerbils, we additionally tested the influence of contralateral stimulation with pure tones to determine the frequency specificity of the CAS effect on the f2–f1 DPOAE. In each animal the primary-tone levels were chosen such that high f2–f1 DPOAE levels were measurable that could be influenced by contralateral noise stimulation (see earlier text). In all four gerbils, contralateral stimulation with pure tones predominantly enhanced the f2–f1 level. The mean induced f2–f1 DPOAE level shift varied between −0.8 and 4.9 dB for the CAS frequencies investigated (0.5–19.03 kHz). The f2–f1 DPOAEs, generated during two-tone stimulation at f2 = 7 and f1 = 5.47 kHz, were maximally affected with CAS frequencies close to 4 kHz (~0.8 octaves below f2; Fig. 5A). For higher

**FIG. 3.** DPOAE growth functions in the presence or absence of contralateral noise stimulation. In the 2 examples (A, B) of DPOAE growth functions, black lines indicate the level of f2–f1, gray lines the level of 2f1–f2. The DPOAE levels are shown before (dashed lines) and during CAS of 50 dB SPL (solid lines). Light gray dots indicate the mean background noise floor.

**FIG. 4.** Averaged data of the effect of contralateral noise on DPOAEs. DPOAE level shift during (A) and after (B) CAS in relation to the period before CAS. Depicted are mean values of f2–f1 and 2f1–f2 level shift ± SE (for CAS = 20–60 dB SPL, n = 12; for CAS = 10 and 70 dB SPL, n = 11). Primary levels were L1 = L2 = 30–55 dB SPL (see text). Asterisks indicate significant difference from zero (Wilcoxon signed-rank test, *P < 0.05, **P < 0.01, ***P < 0.001).
and lower contralateral frequencies the effect strength decreased. This frequency-dependent pattern appeared for all CAS levels tested and the effect strength increased with higher CAS levels (Fig. 5A). To test whether the observed frequency specificity depends on the primary frequencies and thus the DP generation site in the cochlea, in one animal DPOAEs were additionally evoked at lower primary frequencies of f2 and lower contralateral frequencies the effect strength decreased. This frequency-dependent pattern appeared for all  

**Low-frequency biasing effects on f2–f1 DPOAE level: experimental approach**

The f2–f1 distortion level is a sensitive monitor of changes of the OP of the nonlinear transfer function, if the OP is situated close to the point of symmetry (Fig. 1B). To provide evidence that the observed f2–f1 DPOAE level alterations induced by CAS indeed reflect changes of the cochlear amplifier OP, we acoustically biased the cochlear partition ipsilaterally by a low-frequency tone to produce periodical operating point shifts. Within the range of used bias-tone levels (89–109 dB SPL), low-frequency biasing resulted in a phase-related f2–f1 amplitude modulation, whereas 2f1–f2 was much less affected. Figure 6 shows three examples of such phase-related DPOAE level modulations induced by the low-frequency bias tone. The shape of the f2–f1 amplitude modulation pattern consisted either of a single modulation with one distinct minimum and one maximum during one bias phase cycle or of a double-modulation pattern with a second minimum and maximum. During the period of CAS, the bias-induced modulation pattern clearly changed. In the examples shown in Fig. 6, the pattern changed from the double-modulation type to the simple-modulation type (Fig. 6, A and B) or vice versa (Fig. 6C). The latter behavior appeared in a case where CAS produced the atypical f2–f1 suppression instead of enhancement during the basic experiments without biasing (see e.g., Fig. 3B).

To evaluate the impact of both low-frequency biasing and CAS on DPOAE, series of measurements with increasing bias tone levels but constant CAS levels (see following text, Fig. 7) and series with increasing CAS levels but constant bias tone levels (see following text; Fig. 8) were carried out and the data were compared with simulation data (Fig. 1).

**Impact of CAS on the f2–f1 modulation pattern induced by bias tones with increasing level**

The strength and type of the f2–f1 amplitude modulation pattern induced by low-frequency biasing depended on the level of the bias tone: low bias levels induced a weak f2–f1 amplitude modulation with one minimum and one maximum (“single modulation”); higher bias levels yielded a stronger f2–f1 modulation with a second maximum (“double modulation”). At very high bias-tone levels, in addition to the f2–f1 DPOAE, the amplitude of the 2f1–f2 DPOAE was also slightly modulated. Figure 7, A and B illustrates the dependence of the amplitude modulation pattern on the bias tone level and additionally show the changes in the modulation pattern induced by CAS. During contralateral noise application, the f2–f1 modulation pattern changed considerably. This is obvious particu-
larly at bias tone levels that already evoked a double-modulation pattern, which switched to a single-modulation pattern during CAS (Fig. 7, A and B; bias tone levels: 101–103 dB SPL; see also Fig. 6). In all eight gerbils tested, comparable systematic changes in the f2–f1 modulation pattern were observed during increasing bias tone levels.

The experimentally observed changes of the biasing induced f2–f1 modulation patterns could be simulated by the Boltzmann function model: increasing the bias level in the simulation produced pattern changes that were comparable to the changes observed in the experiments (Fig. 7, B and C, dotted lines). An additional shift of the initial OP in the simulation, mimicking CAS-dependent changes of the OP, induced a distinct pattern change (Fig. 7C, solid lines) that was also observed during the period of CAS in the experiments (Fig. 7B, solid lines). A conversion of the double-modulation pattern to a single-modulation pattern, as observed in the majority of experiments, corresponds to a shift of the OP away from the inflection point of the Boltzmann function toward asymmetric positions.

Impact of CAS level on the biasing-induced f2–f1 modulation pattern

To evaluate the strength of CAS-induced effect on the f2–f1 modulation pattern during low-frequency biasing, we systematically increased the CAS level from 20 to 70 dB SPL, whereas the bias tone level was kept constant per experiment (range 99–107 dB SPL). Figure 8, A and B shows the bias-induced f2–f1 modulation patterns before, during, and after CAS with increasing levels in representative test series of two animals. The strength of CAS-induced pattern change correlated with the level of contralateral stimulation and increased to higher CAS levels. Comparable results of increasing modula-
tion pattern changes due to increasing CAS levels were measured in six additional animals.

Figure 8C gives a series of simulated data where the OP was initially positioned \( s_1 = 0.0495 \), such that the modulation pattern resembled the pattern observed in the experiments without CAS (Fig. 8, A and B, dotted lines). Additional shifts of the OP (Fig. 8C, from bottom to top, from \( s = -0.0500 \) to \( s = -0.0530 \)) gradually changed the \( f_2-f_1 \) modulation pattern, reducing the smaller modulation peak at about 90° bias phase. Similar pattern changes could be observed for increasing CAS level in the experimental data (Fig. 8, A and B, solid lines). The OP shifts were quite small, compared with the two OP positions exemplified in Fig. 1, thus indicating that minute OP changes close to the point of symmetry of the Boltzmann function are sufficient to produce the observed effects.

**DISCUSSION**

**CAS-induced efferent effects on \( f_2-f_1 \) and on \( 2f_1-f_2 \) DPOAE levels**

The present study reports a strong amplitude alteration of the quadratic \( f_2-f_1 \) DPOAE during CAS. This influence of CAS on the quadratic DPOAE at \( f_2-f_1 \), which increased for higher CAS levels and was much larger than the effect on the cubic DPOAE at \( 2f_1-f_2 \), can be seen analogous to previous studies that report a stronger effect of CAS on \( f_2-f_1 \) than that on \( 2f_1-f_2 \) (Chang and Norton 1997; Kirk and Johnstone 1993; Kujawa et al. 1995; Wittekindt et al. 2009). Because the effects already occurred at low contralateral noise levels (20 dB SPL), which is far below the middle ear reflex threshold (human: Gelfand 1984; rat: Pilz et al. 1997), a contribution of middle ear muscle reflex to the DPOAE level shifts might be excluded.
The observed effects most likely can be attributed to a CAS-induced activity of the medial olivocochlear (MOC) efferents that innervate the outer hair cells. This is also supported by the calculated time constants of the CAS-induced amplitude change (τ ≈ 55 ms) that matched well with the time characteristics of the fast component (10–100 ms) of the MOC reflex described in studies in cats, guinea pigs, and humans (Backus and Guinan 2006; Cooper and Guinan 2003; Liberman et al. 1996). After contralateral stimulation significant off-effects in the form of a level reduction of the f2–f1 DPOAE were observed (see Figs. 2 and 4). Off-effects are also reported for auditory-nerve fiber activity following a suppression due to efferent stimulation (Guinan and Gifford 1988b), but these may involve the lateral olivocochlear system. Besides these fast MOC effects, slower components exist (Backus and Guinan 2006; Cooper and Guinan 2003; Sridhar et al. 1995) that could alter the DPOAE amplitude on a longer timescale, which are not investigated systematically in our study.

In the present study, the lowest contralateral stimulus level that already induced significant amplitude shifts of the f2–f1 DPOAE was 20 dB SPL, which is clearly less than the CAS level sufficient to induce a significant amplitude shift of the 2f1–f2 DPOAE (40 dB SPL). Further, the strength of the CAS-induced f2–f1 level shift exceeded the effect on 2f1–f2 for all CAS levels investigated. The mean induced f2–f1 level shift was 5.1 dB at the highest CAS level of 70 dB SPL (individual maximal shift: 10.1 dB, at 60 dB SPL CAS), which clearly exceeded the CAS effect on 2f1–f2 in the present study. It should be noted that the primary-tone level ratio (L1 = L2) used here does not correspond to the optimal ratio for 2f1–f2 DPOAE generation in gerbils (Pibal et al. 2002) and that this could affect the sensitivity for efferent modulation. However, both DPOAEs were well measurable under these stimulus conditions.

Until now, there are only few studies investigating the quadratic f2–f1 DPOAE under contralateral sound stimulation (Chang and Norton 1997; Kirk and Johnstone 1993; Kujawa et al. 1995; Wittekindt et al. 2009) or generally during activation of the olivocochlear efferents (e.g., Mountain 1980; Siegel and Kim 1982). Most of these studies report a level reduction of f2–f1 due to efferent manipulations or bipolar effects. In our study, the typical behavior was a f2–f1 enhancement, although we also found reduction of f2–f1 in a minority of cases. Level decrease versus enhancement may vary in dependence on the position of the operating point of the cochlear amplifier and on the primary-tone level, which could be different for different preparations. Concerning the 2f1–f2 DPOAE, typical level reductions during CAS are in the range of 0.5–4 dB and are induced by CAS levels of 25–70 dB SPL (various species; e.g., Kujawa et al. 1993; Lisowska et al. 2002; Moulin et al. 1993; Puel and Rebillard 1990). In comparison, the f2–f1 changes are larger and can be induced by lower CAS levels, which indicates that the f2–f1 DPOAE might be a more sensitive monitor of CAS-induced alterations of the cochlear function than the typically used 2f1–f2 DPOAE.

The influence of the primary-tone level on the CAS-induced DPOAE level alterations was investigated systematically by measuring DPOAE growth functions with and without CAS. Typically the f2–f1 DPOAE showed a level enhancement during CAS for low and moderate primary levels. Especially in the notch region of the growth function, the CAS-induced level shift of the DPOAE could be huge and the direction of the shift could be inverted. This phenomenon of a shifted DPOAE growth function due to efferent activity, which results in atypical effects in the notch region and both suppression and enhancement in dependence on the primary-tone level, was also described for DPOAE (2f1–f2) onset behavior (Kujawa and Liberman 2001; Lukashkin and Russell 2002; Maison and Liberman 2000), which is attributed to olivocochlear efferent activity evoked ipsilaterally by the primary tones. Furthermore, Müller et al. (2005) reported bipolar effects on DPOAE level induced by CAS in humans. At high primary-tone levels, the CAS effect no longer occurred, which is in good agreement with the idea that efferent modulation of cochlear amplification predominantly acts at low sound levels and is in accordance with described CAS effects on DPOAE (2f1–f2) growth functions (Janssen and Gehr 2003; Moulin et al. 1993).

Frequency specificity of the efferent system

The experiments using pure tones as CAS (Fig. 5) clearly show that the efferent modulation acts in a frequency-specific manner. Maximal f2–f1 level shifts could be measured when the contralaterally applied sinusoids had frequencies about 0.8 octaves lower than the ipsilateral primaries (f2 = 7 kHz). The frequency specificity is also evident when comparing the DPOAE level shifts obtained for different primary and various CAS frequencies (Fig. 5B). Here a clear shift of the maximal CAS-induced level shift toward lower CAS frequencies can be observed for the lower primary frequency combination (f2 = 5 kHz). Although the presented data are preliminary (a detailed study is in preparation) and a final conclusion requires further investigations, our observation is consistent with the DPOAE study of Chang and Norton (1997), who found maximal impact on the f2–f1 DPOAE level with CAS frequencies half an octave below the f2 frequency, and with a recent study of Lilanöytikul and Guinan (2009) who describe maximal effects on DPOAEs with contralateral elicitor frequencies 0.5–1 octave below the probe frequency. The frequency-specific effects are in agreement with the course of anatomical projections of the MOC efferents in the cochlea (Guinan et al. 1984), suggesting that the MOC neurons project to a position more basally compared with their characteristic frequency.

Low-frequency biasing of the cochlear partition

The difference tone f2–f1 is more sensitive to changes of the OP of the cochlear amplifier transfer function than the 2f1–f2 DPOAE. This was shown in previous studies and in the present study using low-frequency biasing (Bian 2004; Frank and Kössl 1996, 1997; Lukashkin and Russell 2005). The pronounced changes in the f2–f1 level during CAS could therefore be interpreted as an efferent-induced shift of the OP of the cochlear amplifier transfer function.

This interpretation is backed by the results of the second part of this study, where we combined the DPOAE measurements during CAS and the method of low-frequency biasing. The low-frequency tone induced a specific f2–f1 modulation pattern, which was characterized by a single-peak or a double-peak modulation, and changed systematically with the level of the bias tone. Further, the observed modulation pattern of the f2–f1 DPOAE is much stronger than the modulation pattern of
2f₁–f₂. This is comparable with the results of previous studies (Bian 2004; Frank and Kössl 1996, 1997; Lukashkin and Russell 2005), where either acoustical biasing with a low-frequency tone or biasing by direct electrical stimulation of the hair cells was used. Further, the changes in DPOAE modulation patterns in dependence on the bias tone level (e.g., Fig. 7) are reminiscent of changes in the AC receptor potential of outer hair cells during low-frequency biasing (Russell and Kössl 1992). In this in vivo study in guinea pigs, a shift of the operating point of outer hair cells associated with the hair cell DC potential was suggested as the driving force for the observed amplitude and phase changes of receptor potentials.

The additional application of contralateral noise produced pronounced changes in the DPOAE modulation pattern that was induced by low-frequency biasing and the strength of pattern change was correlated with the CAS level. According to our simulation, the CAS-induced change of the modulation pattern is consistent with a shift of the OP of the transfer function and increasing CAS levels would produce increasing OP shifts. Thus the observed change of the modulation pattern during CAS might be evoked by a shift of cochlear amplifier OP during CAS-induced efferent activity.

In our Boltzmann function simulation, the initial OP is located on the negative side of the inflection point and the efferent-induced shift was in the negative direction, away from the inflection point. Similar results in terms of changes of the modulation pattern would be obtained if the initial OP is located on the positive side of the inflection point and an efferent-induced shift is in the positive direction because the f₂–f₁ DPOAE level is minimal at the inflection point and shifting the OP away from the inflection point, regardless of the shift direction, will produce f₂–f₁ DPOAE level enhancement as observed in our experiments. Of course, the comparison between DPOAE modulation patterns in the experimental data and the simulation provides only a qualitative assessment and real values for efferent shifts of the OP have to be obtained by other methods. Furthermore, the observed effects on f₂–f₁ DPOAE level could also be influenced by changes in the gain of the cochlear amplifier (Russell and Lukashkin 2008).

**Does the olivocochlear efferents change the OP of cochlear amplification?**

The concept that the olivocochlear efferents are acting through a shift of the operating state of the transfer characteristics of the cochlear amplifier was previously suggested by other authors (Kim et al. 2003; Russell and Lukashkin 2008). However, Patuzzi and Rajan (1990) speculated on the basis of their experiments that shifts of the operating point position are too small to influence cochlear amplification in a way that could explain the efferent effects.

Shifts of the OP due to efferent activity could be based on changes of the mechanoelectrical properties of the outer hair cells and calcium-mediated structural changes (Frolenkov et al. 2003). The functional consequence of an OP shift produced by MOC could be an adjustment of the cochlear amplifier gain. This MOC-induced gain reduction of the cochlear amplifier would correspond to the described damping influence of the MOC on the cochlear amplification (for reviews, see Guinan 1996; Russell and Lukashkin 2008), which is seen in DPOAE studies (see previous text), measurements of basilar membrane motion (Cooper and Guinan 2003; Murugasu and Russell 1996; Russell and Murugasu 1997), and auditory nerve activity (Gifford and Guinan 1983; Guinan and Gifford 1988a,b). The latter studies investigating auditory-nerve rate level functions during electrical stimulation of the crossed olivocochlear bundle see a suppressive effect of the efferents. Such a suppressive effect on auditory-nerve activity was also observed during low-frequency biasing by Schmiedt (1982). Comparable effects of efferent stimulation and low-frequency biasing, which both could shift the operating point of the cochlear amplifier transfer function, is also seen for spontaneous otoacoustic emissions (SOAEs); both efferent influence in terms of CAS and low-frequency biasing affected the SOAE by increasing the SOAE frequency and predominantly decreasing the SOAE level (Bian 2008; Bian and Watts 2008; Harrison and Burns 1993; Mott et al. 1989).

As a conclusion, two points should be considered. First, CAS-induced efferent modulation of cochlear mechanical properties can be detected by measuring the quadratic DPOAE f₂–f₁ more sensitive than by measuring only 2f₁–f₂. Of course, this requires the existence of a difference tone with high amplitude, which presumably will not be the case for all frequencies and all species. Second, the results of the present study and the qualitative comparison with the simulated data suggest that efferent effects on DPOAEs might be produced by changing the operating point of the cochlear amplifier.

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**REFERENCES**


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