JARO 02: 268–278 (2001)
DOI: 10.1007/s101620010047

JARO
Journal of the Association for Research in Otolaryngology

Effects of Olivocochlear Feedback on Distortion Product Otoacoustic Emissions in Guinea Pig

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Received: 3 May 2000; Accepted: 6 April 2001; Online publication: 1 August 2001

ABSTRACT

Activation of ipsilaterally responsive olivocochlear (OC) neurons by sound produces rapid, post-onset alterations in the $2f_1–f_2$ distortion product otoacoustic emission (DPOAE). The present study investigates the frequency and level dependence of this ipsilateral OC effect in the anesthetized guinea pig, compares its magnitude and sign to OC effects elicited by contralateral sound ("contralateral" OC effect), and characterizes the influence of such activity on steady-state DPOAE amplitude. DPOAEs were measured with fine time resolution in response to primary stimuli varied systematically in frequency and level. DPOAEs showed rapid and remarkably stereotyped post-onset amplitude alterations. These ipsilateral OC effects were greater for high (8–12 kHz) than for low (2–4 kHz) f_2 primary frequencies and for higher primary levels (70–80 dB SPL). For any f_2/f_1 pair, the sign as well as the magnitude of the ipsilateral effects varied with primary level ratio. For example, with L_1 fixed at 75 and L_2 varied in 1-dB steps from 60 to 75 dB SPL, DPOAE amplitude underwent a stereotyped progression from post-onset increases at the lowest levels of the f_2 primary to post-onset decreases at the highest levels. At intermediate levels, near the region of sign change (L_2 = 5–10 dB below L_1), post-onset effects were often particularly large (as great as 20 dB). These large ipsilateral OC effects were always associated with "dips" in the DPOAE amplitude vs. level functions, and both disappeared after OC section. Although smaller in magnitude, contralateral OC effects were identical to ipsilateral effects in frequency and level dependence and in form.

Keywords: olivocochlear, otoacoustic emissions, outer hair cells, guinea pig, distortion products, reflex

INTRODUCTION

Activation of olivocochlear (OC) neurons by sound alters cochlear responsiveness as part of a sound-evoked feedback pathway to the inner ear. Conventionally, assays of OC reflex activity assess the effects of contralateral sound on an ipsilaterally monitored cochlear response (e.g., Liberman 1989; Warren and Liberman 1989; Mott et al. 1989; Puel and Rebillard 1990; Kujawa et al. 1993, 1994). Effects monitored with such assays are mediated by contralaterally responsive OC neurons, presumably the medial (M)OC subset, which comprise roughly one-third of the MOC input to a given cochlea (Liberman 1988; Warren and Liberman 1989).

Efferent mediation of the cochlear response to sound also occurs through activation of the more numerous, ipsilaterally responsive OC neurons. In Figure 1, the distortion product otoacoustic emission (DPOAE) at $2f_1–f_2$ shows an OC-mediated post-onset amplitude alteration within several hundred milliseconds of stimulus onset and thereafter reaches a steady-state value (see also Liberman et al. 1996; Kujawa and Liberman 1997, 1999). The OC neurons responsible for this rapid, onset effect are activated by the primary stimulus tones (f_1, f_2) themselves. The DPOAE amplitude can, of course, be perturbed from this steady state by introduction of an additional sound to the

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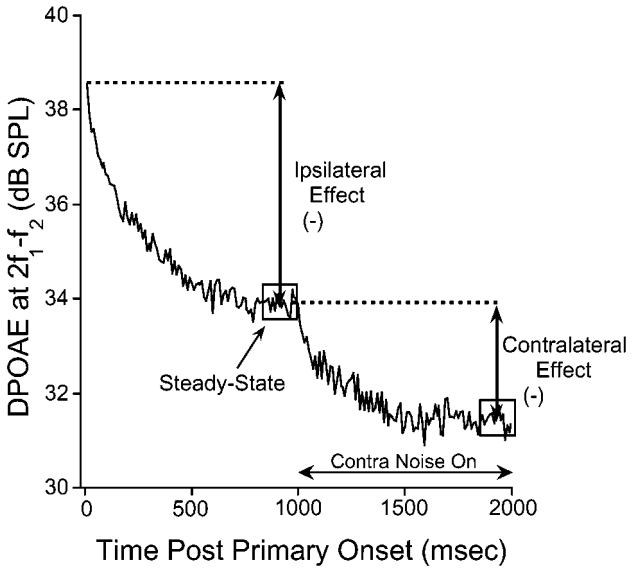

FIG. 1. Amplitude of the $2f_1-f_2$ DPOAE plotted as a function of time after onset of the primary tones (f_2 = 10 kHz at 70 dB SPL; f_1 = 8.33 kHz at 75 dB SPL). DPOAE amplitude declines rapidly to a quasi-steady state, which is maintained until the addition of a contralateral noise (70 dB SPL wideband rms). The noise causes a further rapid decline in the DPOAE to a second steady state. The "Ipsilateral Effect" is defined as the difference in DPOAE amplitude between the average "steady-state" value (calculated over 10 consecutive points immediately before addition of the contralateral noise) and the first post-onset measure ($t \sim 10$ ms). Thus, the effect in this example has a negative sign (—). The "Contralateral Effect" is defined as the difference in steady-state DPOAE amplitudes recorded before and after introduction of the noise.

contralateral ear. When this occurs, the DPOAE again undergoes a rapid amplitude adjustment which mirrors the ipsilateral sound effect in its onset time course. A new steady-state value is reached and is maintained until stimulus conditions are again altered.

DPOAEs have become important tools in the clinical evaluation of cochlear function. Because most sensorineural hearing losses are sensory in nature, otoacoustic emissions are widely promoted as valuable tools in the identification and characterization of peripheral hearing loss (e.g., Norton 1993; Gorga et al. 1997; Kimberly et al. 1997). The premise of this use is that, when middle-ear function is normal, DPOAEs are sensitive metrics of the functional status of the cochlea, in particular, of the outer hair cells (OHCs) (e.g., Siegel et al. 1982; Brown et al. 1989; Liberman et al. 1997). As routinely monitored in clinical applications, the DPOAE response is identified as a spectral peak of appropriate frequency (e.g., $2f_1-f_2$) sitting in a background of "noise". Signal averaging is required to extract these small acoustic signals from the other sounds detected by the recording microphone. Averaging, however, takes time; thus, responses typically are monitored as steady-state amplitudes spanning a number of seconds after stimulus onset.

It is clear that steady-state DPOAEs can be influenced by variables other than peripheral hearing loss. Our focus in this report is the influence of ipsilaterally evoked OC feedback (see also Kujawa and Liberman 1999), i.e., OC activity evoked by the primary tones themselves. In these experiments, the stimulus frequency and level dependence of this ipsilateral OC effect on the $2f_1-f_2$ DPOAE is investigated in anesthetized guinea pigs with normal cochlear function. The magnitude and sign of these ipsilateral OC effects are compared with those of the OC effects elicited by contralateral sound (contralateral OC effect) and the influence of such activity on steady-state DPOAE amplitude is described.

The extent to which OC feedback alters steady-state DPOAEs has important implications for use of these responses as simple metrics of OHC function. Moreover, the dramatic dependence of ipsilateral and contralateral OC effects on primary level and frequency demonstrated here suggests that DPOAE-based assays of OC function may provide a spurious view of OC reflex strength if such parameters are not carefully considered and controlled.

METHODS

Experimental animals

The albino guinea pigs used in this study are a subset of the control group (24 ears of 18 animals) for a study on conditioning-related protection from acoustic injury (Kujawa and Liberman 1997, 1999). They were obtained from the breeder at 325–350 g and were held in a quiet room (ambient SPL ~ 50 dB) without treatment until undergoing the acute physiologic experiments 32 days later. Each animal was then anesthetized (Nembutal, 25 mg/kg IP; Innovar Vet, 0.5 mL/kg IM) and surgically prepared for the recording of compound action potentials (CAPs) of the auditory nerve and distortion product otoacoustic emissions (DPOAEs). Animals described here are those from which detailed measures of ipsilateral and contralateral sound-evoked effects on DPOAEs were obtained. As detailed elsewhere (Kujawa and Liberman 1997, 1999), CAP and DPOAE isoresponse ("threshold") and growth functions were obtained for both ears of all animals across a broad range of frequencies. All findings for this group were consistent with normal cochlear function. All procedures were conducted in accordance with National Institutes of Health guidelines and were approved by the Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary.

Stimulus generation and response recording

Distortion products at $2f_1–f_2$ were measured with an Etymotic Research (ER), (Elk Grove Village, IL, USA) 10C acoustic system inserted into the cartilaginous ear canal. Primary tones (f_1 and f_2, $f_2/f_1 = 1.2$) were generated digitally (20 μs sampling) using a D–A board (AO-6; National Instruments, Austin, TX, USA) in a Macintosh computer operating under LabVIEW control. Ear-canal sound pressure was detected via the low-noise microphone in ER10C probe. The microphone output was passed through a programmable gain amplifier to an A–D board (A-2000; National Instruments) for digitization (20 μs sampling). Microphone sensitivity as a function of frequency was measured before each experiment using a calibrated condenser microphone (Bruel and Kjaer 1/4 in.). Earphone output was calibrated at the start of each measurement. Noise floors varied with stimulus frequency and ranged between −20 and −10 dB SPL.

Ipsilateral OC effects were elicited by the primary tones themselves. These effects were revealed by measuring post-onset changes in DPOAE amplitudes with fine (10 ms) time resolution. Contralateral OC effects were elicited by presentation of a wideband noise (~50 kHz bandwidth; 70 dB SPL) to the opposite ear. Primary tones were presented in 2-s bursts, with contralateral noise introduced 1 s after primary onset. Off-time between primary bursts was 3.5 ms. Responses to 3 stimulus presentations were averaged at each primary level ratio. The ipsilateral ear-canal sound pressure was digitized and broken into contiguous 10.24-ms samples. An FFT was computed on each waveform sample and the amplitude of the $2f_1–f_2$ DPOAE extracted and plotted as a function of post-onset time (Fig. 1). Three values were obtained from such records: (1) ipsilateral OC effect, (2) contralateral OC effect, and (3) steady-state DPOAE amplitude. The "ipsilateral OC effect" was defined as the difference in DPOAE amplitude between the average DPOAE for the 10 points immediately before contralateral noise presentation and the first post-onset measure ($t \sim 10$ ms). The "contralateral OC effect" was defined as the difference in steady-state DPOAE amplitudes recorded before and after introduction of the noise.

Measurements described above were obtained for f_2 = 2, 4, 6, 8, 10 and 12 kHz. Two level protocols were employed: a 5-dB protocol and 1-dB protocol. The 5-dB protocol was designed to screen for the frequency and level regions of maximum ipsilateral and contralateral OC effects. For each primary frequency pair, the level of f_1 (L_1) was incremented in 5-dB steps from 60 to 80 dB SPL with the level of f_2 (L_2) 5 dB < L_1. The 1-dB protocol better characterized the influence of primary level on the magnitude and sign of the OC effects. For this protocol, L_1 was fixed (70, 75, or 80 dB SPL) and at each L_1, L_2 was varied from L_1 − 15 to L_1 in 1-dB steps.

In a subgroup of animals (n = 8), DPOAEs were measured before and after acute section of the OC bundle. A small section of occipital skull was cleared of skin and muscle and was removed, exposing the cerebellum. The cerebellum was then elevated gently, revealing the floor of the IVth ventricle (in some animals, a portion of the middle cerebellar vermis was aspirated to improve the view). "Baseline" (precut) DPOAE measures were repeated at frequency and level combinations of maximum effect (usually f_2 = 8 or 10 kHz and L_1 = 75 dB SPL). An anterior-to-posterior cut was then made in the dorsal surface of the brainstem with a small, sickle-shaped knife and measurements were repeated. The cut was positioned laterally to sever the entire OCB (crossed and uncrossed components) to one ear (Liberman 1990; Kujawa and Liberman 1997).

RESULTS

Variations in ipsilateral and contralateral effects with primary frequency and level

As illustrated in Figure 1, $2f_1–f_2$ DPOAEs recorded in guinea pig undergo rapid post-onset amplitude changes (Kujawa and Liberman 1997, 1999), before reaching a quasi-steady state within 1 s after primary onset. This ipsilateral effect is usually well fit by a single exponential with a time constant within the range 100–250 ms. The onset time constant for the ipsilateral effect is similar to that seen for DPOAE amplitude change induced by addition of contralateral sound, i.e., the "contralateral" effect. Although the ipsilateral and contralateral post-onset effects shown in Figure 1 resulted in DPOAE amplitude *reductions*, ipsilateral and contralateral effects also can *increase* DPOAE amplitudes (Liberman et al. 1996; Kujawa and Liberman 1997, 1999; see also Fig. 4). Both ipsilateral and contralateral effects, whether positive or negative in sign, largely disappear when the OC bundle is cut (see below); thus, the effects appear to be OC-mediated.

Using a 5-dB protocol for incrementing primary level (L_2 = 5 dB < L_1), ipsilateral and contralateral effects were sampled across a broad range of frequencies (2–12 kHz) and levels (55–80 dB SPL) of primary stimulation. In Figure 2, each point reflects the ipsilateral and contralateral effect magnitudes for one combination of primary frequency and level. Across the range sampled, the magnitude of the ipsilateral effect was proportional to (and typically twice as large as) the contralateral effect. The "sign" of the effects was

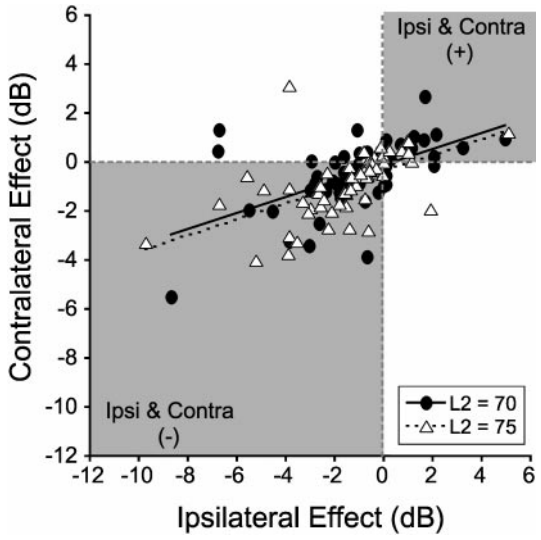

FIG. 2. For a wide range of primary frequencies and levels, the magnitude of the ipsilateral effect is proportional to that of the contralateral effect. The "signs" of the effects usually are the same. Each point represents the ipsilateral and contralateral effect magnitudes for one combination of primary frequency and level. Effect magnitude and sign were computed as described in Fig. 1. f_2 was at 2, 4, 6, 8, 10, or 12 kHz. L_2 was either 70 or 75 dB SPL (see key), and L_1 was always 5 dB above that of f_2. The solid and dashed lines are best fits to the data at 70 and 75 dB, respectively.

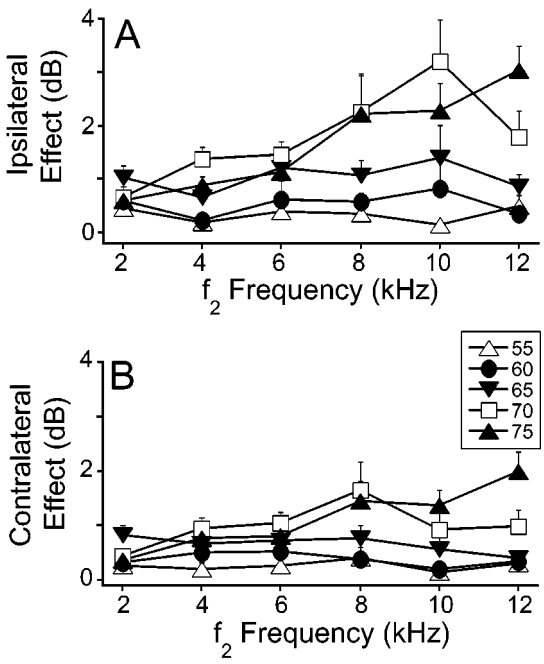

FIG. 3. Frequency and level dependence of ipsilateral and contralateral effects as revealed by the 5-dB "screening" protocol. Data represent average ipsilateral (**A**) and contralateral (**B**) effect magnitudes seen in this sample of ears ($n = 24$). The data at 70 and 75 dB are the same as those in Fig. 2. At each f_2 frequency (2, 4, 6, 8, 10, or 12 kHz), primaries were presented at 5 different sound pressure levels: L_2 is shown in the key; L_1 was always 5 dB higher. For this analysis, ipsilateral and contralateral effects were defined as shown in Fig. 1; however, to eliminate polarity differences, the absolute values were computed before averaging. Error bars represent standard errors of the means.

almost always the same. That is to say, when the post-onset adaptation caused a DPOAE amplitude reduction (classified as a negative ipsilateral effect), the addition of contralateral noise also decreased the DPOAE. Correspondingly, when the post-onset adaptation increased the DPOAE, addition of contralateral sound also increased the DPOAE. The ipsilateral and contralateral effects also were similar functions of frequency and level of stimulation. Absolute values of these effects were averaged across ears and are displayed in Figures 3A and B. For all animals tested, effects were most robust at higher frequencies (8–12 kHz) and higher levels (70–80 dB SPL) of primary tone stimulation.

Variations in ipsilateral and contralateral effects with primary level ratio

To investigate more carefully the dependence of effect magnitude on primary level ratio, a 1-dB protocol was used. Here, L_1 was fixed (70, 75, or 80 dB), and at each L_1, L_2 was varied from $L_1 - 15$ to L_1 in 1-dB steps. In most animals, effect magnitudes grew as L_1 was increased from 70 to 75 dB SPL; however, effects at 80 dB SPL were similar to or slightly smaller than those at 75 dB SPL.

Effect magnitudes at these high frequencies and levels of stimulation were exquisitely sensitive to primary level ratio. Raw data from one animal are shown in Figure 4. In this example, L_1 was fixed at 75 and L_2 was varied in 1-dB steps from 60 to 75 dB SPL; for clarity, data for the 16 level ratios are plotted across three panels. The post-onset effect undergoes a progression from increases (ipsilateral effect positive) at the lowest values of L_2 (Fig. 4A) to decrease (ipsilateral effect negative) at the higher (Fig. 4C). At intermediate levels (Fig. 4B), the sign of the effect changes from positive to negative and the magnitude of the effect is maximum. In cases where the ipsilateral effect was particularly large, the sign change was sometimes biphasic, occurring over a 2-dB span of L_2 as shown in this example. Contralateral effects mirror the ipsilateral effects in their level dependence; they were proportionately smaller but usually of the same sign. Only within the intermediate level regions (as shown in Fig. 4B) were ipsilateral and contralateral effects ever different in sign: in such cases, the L_2 value at sign change differed by at most 2 dB for ipsilateral vs. contralateral effects.

This relationship between effect sign/magnitude and primary level ratio was remarkably stereotyped across animals. Data from a number of different animals are superimposed in Figure 5. Consider first the

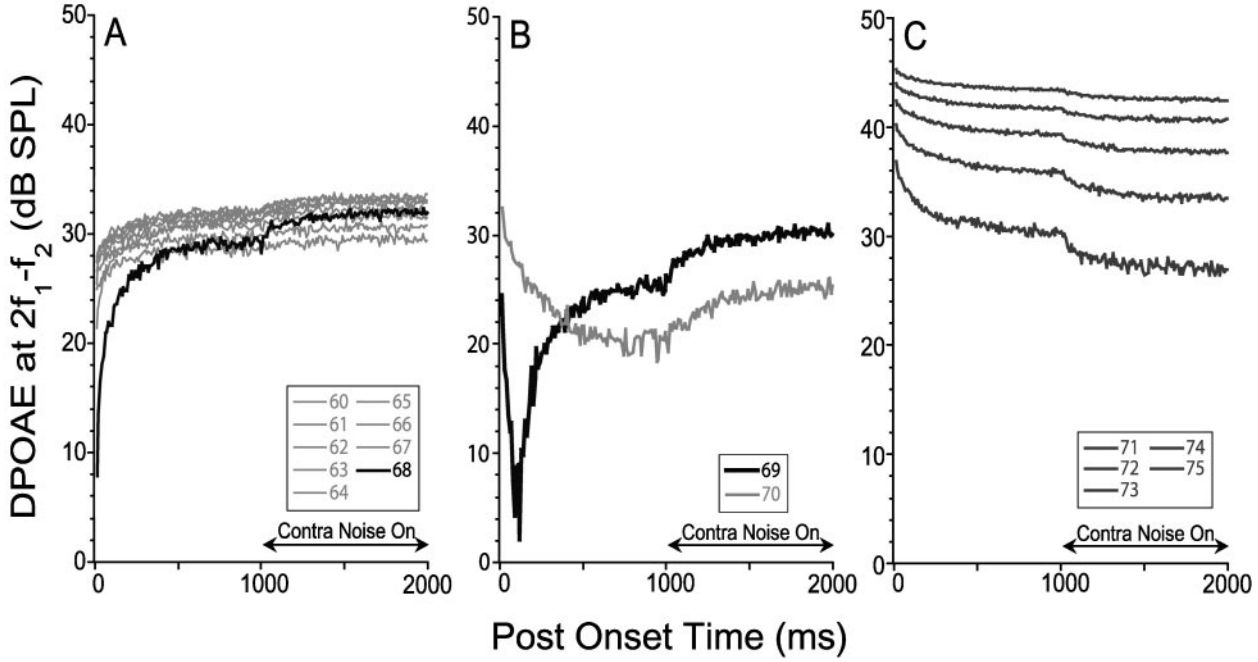

FIG. 4. Effect of varying f_2 level on the magnitude and sign of post-onset adaptation and contralateral sound effects. Data from all three panels are from the same ear and represent consecutive runs all obtained within roughly 5 minutes of each other. The ipsilateral stimuli were two primary tones of 2000 ms duration: f_2 was at 10 kHz, f_1 was at 8.33 kHz at 75 dB SPL, and f_2 level varied in 1-dB steps from 60 to 75 dB SPL. Results from the 16 different f_2 levels are presented in three different panels for the sake of clarity. The contralateral stimulus was always a broadband noise at 70 dB SPL, turned on at t = 1000 ms during each run. **A**. At lower f_2 levels, rapid onset changes in DPOAE amplitude are positive (i.e., steady-state amplitudes are increased by the ipsilateral and contralateral OC effects). **B**. As L_2 is incremented through intermediate levels, ipsilateral and contralateral effects grow dramatically and ultimately change sign. **C**. As L_2 continues to be incremented to higher levels, effects remain negative in sign (steady-state DPOAEs are made smaller by the effects).

ipsilateral effects (Figs. 5A–D) in which effect magnitude is plotted vs. L_2 (L_1 fixed at 75 dB SPL). As discussed above, ipsilateral effects for low-frequency primaries (2 kHz, 4 kHz; Figs. 5A and B) were small. In contrast, ipsilateral effects for high-frequency primaries (8 kHz, 10 kHz; Figs. 5C and D) can be large, especially for level ratios where L_2 is 5–10 dB less than L_1. In this range, the ipsilateral effect grows to large positive values, flips abruptly to large negative values, and then wanes in size with further increases in L_2. Contralateral effects for these same ears and conditions of stimulation (Figs. 5E–H) were similar to ipsilateral effects in frequency and level dependence, although they were smaller in overall magnitude. Moreover, contralateral effects also mirrored ipsilateral effects in the dependence of sign on L_2.

Steady-state DPOAEs are shaped by ipsilateral effects

The steady-state DPOAE amplitudes appear to be influenced by the magnitude of the OC effects. Level ratios associated with the largest ipsilateral and contralateral effects are also associated with local minima in the steady-state DPOAE amplitudes. This relationship is clearly evident in the data from the bottom row of Figure 5, in which the steady-state DPOAE amplitudes have been extracted from the same ears and conditions illustrated in the upper two rows. In low-frequency regions where OC effects are small, DPOAE amplitudes grew monotonically with increasing L_2. In contrast, at high primary frequencies, steady-state amplitudes show prominent "dips" in the L_2 level functions in precisely the same region where the OC effects grow and change sign.

Ipsilateral and contralateral sound effects on DPOAEs are OC-mediated

Rapid, post-onset alterations in the $2f_1$–f_2 DPOAE disappear almost completely upon cutting the OC bundle in the brainstem. In Figure 6A, onset effects are highlighted for several stimulus level combinations yielding particularly robust changes in the DPOAE amplitude. After OC section (Fig. 6B), these onset effects are dramatically reduced. The inset shown in panel B takes one of the post-cut traces and increases the gain to more clearly display the onset transient remaining after the cut.

Effects of OC section on ipsilateral and contralateral effects are summarized in Figures 7A and B and on

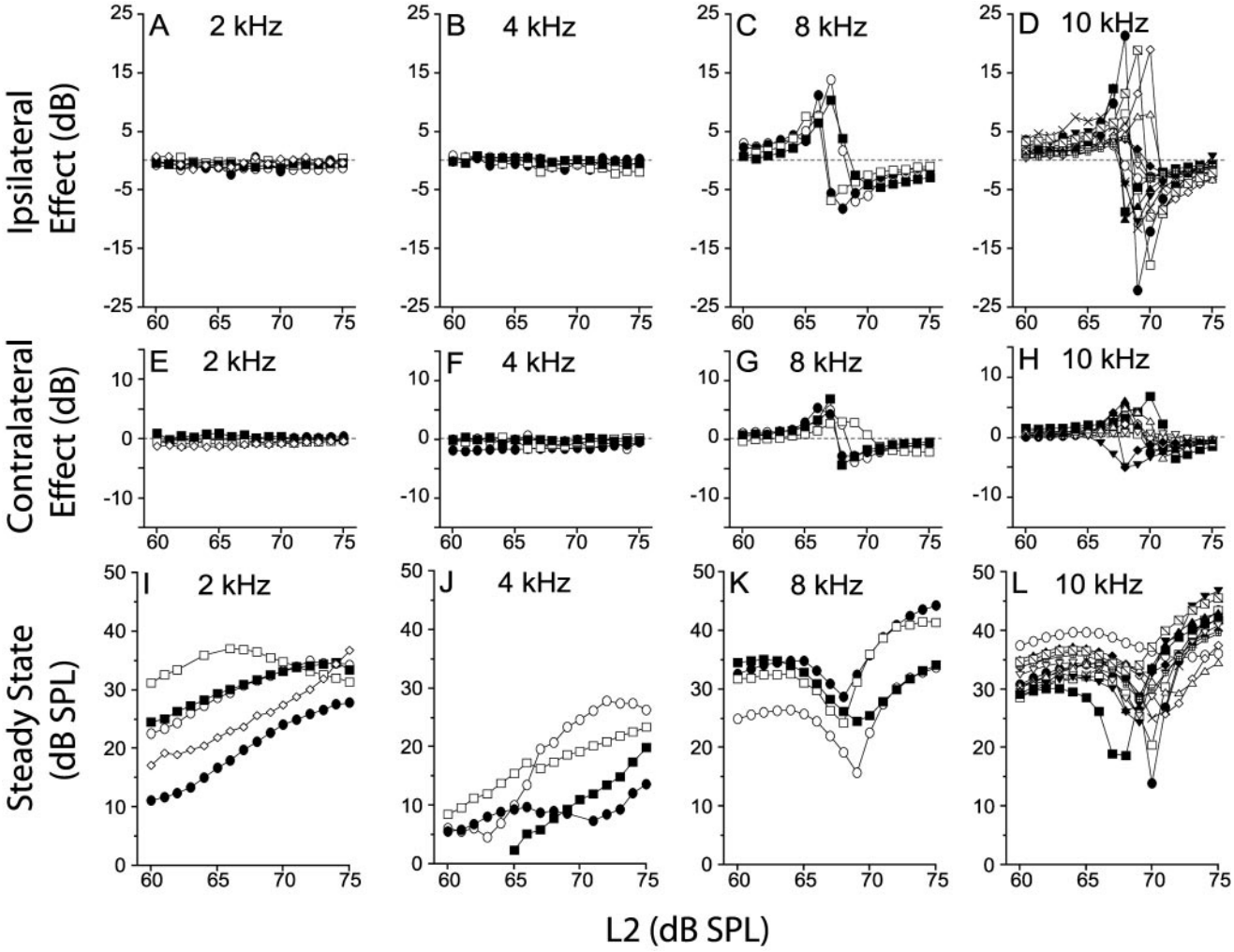

FIG. 5. Effect of varying L_2 on the ipsilateral effect (top row), contralateral effect (middle row), and steady-state DPOAE (bottom row) in a large sample of animals. Data within each panel are superimposed from all animals receiving identical stimulations: f_2 = 2, 4, 8, and 10 kHz, as indicated in each panel; L_1 = 75 dB SPL; L_2 varied as shown on the *x* axis. All values were extracted as described for Fig. 1. For a given ear, ipsilateral and contralateral effects and steady-state amplitudes are displayed using the same symbol. Although OC reflex strength differs between animals, all show (1) a stereotyped progression from positive to negative OC effects, (2) increased effect magnitude in the region of the sign change, and (3) a clear association of ipsilateral effect magnitude and sign with a dip in the DPOAE growth function.

steady-state amplitudes in Figure 7C. As shown previously in the cat (Liberman et al. 1996), a small amount of slow post-onset adaptation to ipsilateral-only stimulation also can remain after the cut and is presumably due to non-OC effects. The prominent "dip" in the steady-state amplitude vs. level function is also greatly diminished by the OC section (Fig. 7C). Note that at some level ratios, the cut can change these steady-state DPOAEs by almost 10 dB, sometimes increasing and sometimes decreasing its amplitude.

DISCUSSION

Mechanisms underlying post-onset adaptation

Liberman et al. (1996) first demonstrated post-onset adaptation of the DPOAE in the anesthetized cat. They suggested that the effect was mediated by ipsilaterally responsive OC neurons. They showed that (1) the time constant of the effect was similar to that seen upon addition of contralateral sound, (2) both time constants (ipsilateral and contralateral) were similar to those seen for other studies of peripheral OC effects, and (3) almost all the post-onset adaptation disappeared upon cutting the crossed OC bundle, the tract known to carry the ipsilaterally responsive medial (M)OC neurons. Since this midline cut fails to sever the bulk of the lateral (L)OC system, the simplest interpretation is that post-onset effects arise from activation of MOC neurons to OHCs.

Post-onset adaptation of the $2f_1$–f_2 DPOAE is also demonstrable in the anesthetized guinea pig (Kujawa and Liberman 1997, 1999). Here we confirm and extend evidence that the effect is MOC-mediated, i.e.,

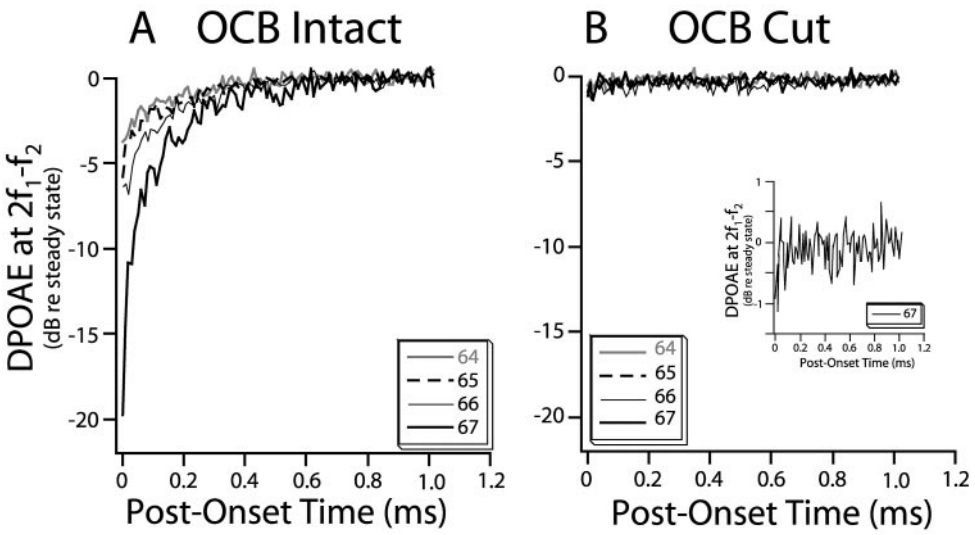

FIG. 6. Amplitude of the $2f_1–f_2$ DPOAE relative to steady state before (**A**) and after (**B**) OC section. Data were recorded for 1000 ms of continuous primary stimulation (f_2 = 8 kHz at 75 dB SPL; f_1 = 6.67 kHz at 64–67 dB SPL) without addition of contralateral sound. The robust onset transients seen in the intact ear are largely absent after acute OC section. The inset in **B** provides an expanded gain view of the effect remaining for one level ratio after the cut.

(1) that the time course of the effect is consistent with known sound-evoked OC effects expressed at the level of single auditory nerve fibers (Warren and Liberman 1989); (2) it is consistent with the level dependence of MOC influences on cochlear responses (Gifford and Guinan 1987; Guinan and Stankovic 1996; Brown et al. 1998); (3) that the effect peaks for primary frequencies near 10 kHz, where the density of MOC terminals on OHCs is greatest (Liberman and Gao 1995) and where single MOC neurons show the highest sound-evoked discharge rates (Robertson and Gummer 1985; Liberman 1988; Brown 1989); and (4) that it largely disappears, along with contralateral effects, when the entire OC bundle is cut (Liberman et al. 1996).

It must be emphasized that, in both the previous and the present studies, post-onset adaptation does not completely disappear upon severing the OC bundle. In a previous study in cat, post-onset effects remaining after OCB section had long time constants (>700 ms), and the pre-cut data were best fit by a double exponential consisting of both fast ($\tau \sim$ 100 ms) and slow components. In the present study, some of the small post-onset effects that survived OC section appeared to be relatively fast ($\tau <$ 250 ms), and the pre-cut data were very well fit by a single exponential. These surviving fast effects were exceedingly small in magnitude, however (see Fig. 6B). The significance of these differences is not yet clear. However, there are clearly other (non-OC-mediated) cochlear mechanisms that cause small post-onset changes in DPOAE amplitude. Although small post-onset adaptations have been described in other species, including the mouse (Sun and Kim 1999), it cannot be safely concluded that they are OC-mediated unless their disappearance after chemical, surgical, or genetic de-efferentation has been documented.

The observed differences in frequency region of maximum ipsilateral effects between cat (f_2 = 2–4 kHz; Liberman et al. 1996) and guinea pig (f_2 = 10–12 kHz, i.e., the highest frequencies tested; see Fig. 3) are consistent with the observed species differences in sound-evoked discharge rates in single MOC neurons. Specifically, in the anesthetized cat, MOC neurons with CFs around 2–4 kHz (Liberman 1988) have the highest sound-evoked discharge rates; whereas in the anesthetized guinea pig, the maximum sound-evoked rates in single MOC neurons grow monotonically with CF (Brown 1989).

It has long been clear that contralateral sound effects on OAEs are mediated by medial (M)OC neurons acting at the level of the outer hair cells. Contralateral sound effects on DPOAEs can be mimicked by intracochlear perfusion of the MOC neurotransmitter acetylcholine (Kujawa et al. 1992), and both the acetylcholine-induced and sound-evoked effects on DPOAEs are blocked reversibly by antagonists of the cholinergic receptor on the OHCs (Kujawa et al. 1993). Indeed, the *in vivo* pharmacology of contralateral sound effects on DPOAEs (Kujawa et al. 1994) is virtually identical to the pharmacology of the nicotinic receptor characterized at the level of *in vitro* OHCs (Erostegui et al. 1994) and subsequently classified as closely resembling the α9 subtype (Elgoyhen et al. 1994). Contralateral sound effects on OAEs also can be prevented by sectioning OC pathways in the brainstem (e.g., Puria et al. 1996). Consistent with sound-evoked activation of OC neurons, direct electrical stimulation of these pathways alters DPOAEs (Mountain 1980; Siegel and Kim 1982); those effects on OAEs can be prevented by pharmacologic blockade (Siegel and Kim 1982), and they are absent in a knockout mouse lacking the α9 receptor (Vetter et al. 1999).

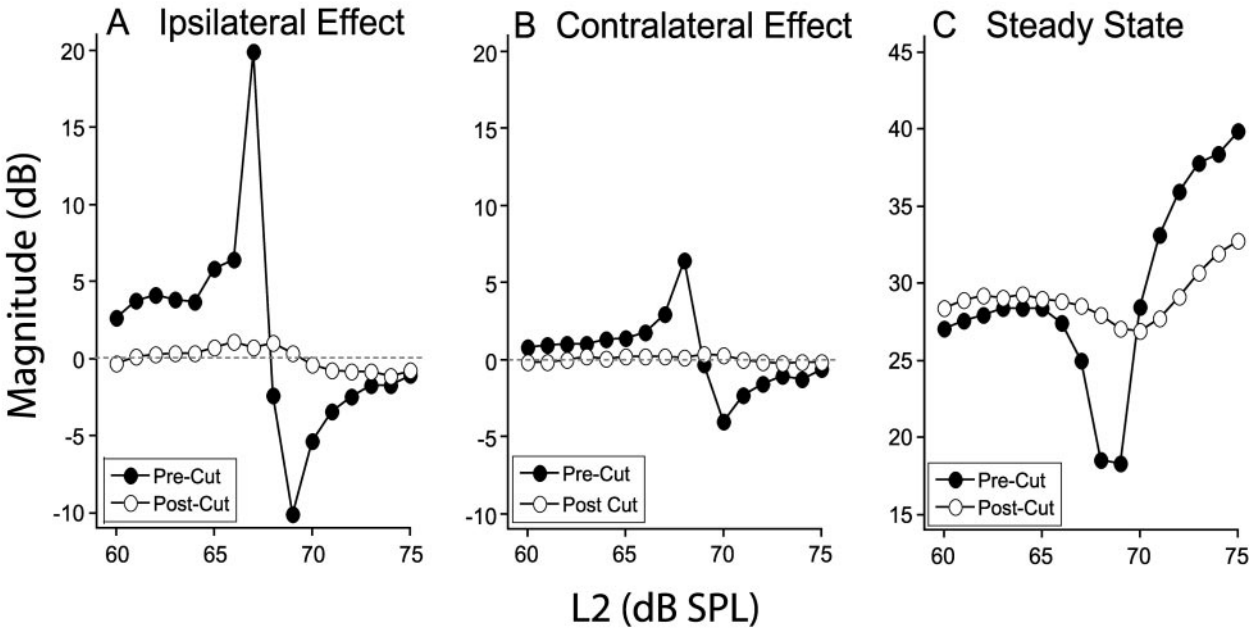

FIG. 7. Effects of OC section on the ipsilateral effect (**A**), contralateral effect (**B**), and steady-state DPOAE (**C**) from one experiment. Pre-cut data were extracted from the same runs shown in Figs. 4A–C (intact). Values obtained after complete unilateral OCB section are shown with dashed lines and open symbols. Before the cut, the progression of ipsilateral and contralateral effects from positive to negative can be seen, as well as the coincidence of the region of sign change with the "dip" in the DPOAE amplitude-vs.-level function.

The observation that both ipsilateral and contralateral OC effects on the DPOAEs can sometimes increase and sometimes decrease the DPOAE amplitude is initially puzzling. It is not, however, unique to the present study or, indeed, to effects of sound-evoked OC activity. Previous work in the cat also reported that sound-evoked OC effects on DPOAEs could change in sign as the level ratio between the primaries was varied (Liberman et al. 1996). Furthermore, an early study of effects of electrically induced OC activity on DPOAEs also noted that amplitudes were sometimes increased and sometimes decreased (Siegel and Kim 1982). Neither previous study systematically investigated this phenomenon. In experiments reported here, the signs of ipsilateral and contralateral effects are almost always the same. Furthermore, the dependence of effect sign on the frequency and levels of the primaries is remarkably reproducible from animal to animal (Fig. 5).

Given the complex nature of the $2f_1–f_2$ DPOAE detected in the ear canal, it is not difficult to postulate, at least in broad outline, how an OC-mediated enhancement of DPOAE amplitude might arise. The ear-canal DPOAE likely comprises two components: (1) a distortion component generated near the f_2 place which travels both basally to the stapes and apically to the $2f_1–f_2$ place where (2) it is amplified and reflected back to the stapes as the second component (Kim 1980; Shera and Guinan 1999). These two components will have phase differences that are frequency and level dependent such that, in the ear canal, they can interact constructively or destructively (see Fahey and Allen 1997 for discussion). Thus, if the two DPOAE components are in partial cancellation in the ear canal and if OC activation affects the two components to differing degrees, the overall effect may sometimes be to enhance the ear-canal distortion product.

The magnitude of the post-onset effects shown here also is initially puzzling. It is unprecedented in the literature on sound-evoked OC effects on otoacoustic emissions to see changes as large as 20 dB. However, post-onset effects of this size are seen routinely in our anesthetized guinea pigs, so long as the frequencies and levels of the primaries are carefully chosen to fall near prominent nonmonotonicities in the DPOAE growth functions. The fact that these large effects disappear immediately and almost completely when the efferent pathways are cut at the brainstem midline suggests that they must be OC-mediated (see Figs. 6 and 7). Such cuts are not close to the facial genua, thus involvement of the stapedius reflex is very unlikely. Furthermore, the fact that they peak for primaries near 10 kHz is not consistent with middle-ear muscle effects in guinea pig (Avan et al. 1992). It is hypothesized that such large effects were observed here because we have identified peculiar, yet highly reproducible, regions in the f_1/f_2 stimulus space in the guinea pig in which cochlear effects of OC activation are essentially amplified. We hypothesize that this amplification takes place for two reasons. First, as discussed above, the steady-state ear-canal DPOAE may reflect partial cancellation of two independent DP

sources in the cochlea near the dips in the growth function. This can enhance the apparent effects of the OC system if the OC feedback affects one component more than the other. Second, it must be considered that DPOAE generation requires the presentation of two stimuli that will interact with each other on the basilar membrane to produce a variety of two-tone suppressive effects. By affecting one component more than another, the effects of the OC system could be amplified by the nonlinear growth of suppression: suppression strength can grow by more than 3 dB for every 1-dB increase in suppressor level (Delgutte 1990).

Use of DPOAEs to assess OC reflex strength

Present results show that post-onset changes in DPOAE amplitude provide a powerful, noninvasive means to study ipsilaterally evoked OC activity. Because results across animals are stereotyped, this assay provides a reliable metric of OC reflex strength. In other work, the assay has been used to evaluate the success of de-efferentation surgeries (e.g., Kujawa and Liberman 1997) and to compare OC reflex strength in animals undergoing conditioning (Kujawa and Liberman 1999) and traumatic noise exposures (Maison and Liberman 2000). This ipsilateral assay has at least two advantages over the conventional contralateral sound-based assay. First, it requires only monaural stimulation. This can be of value when applied to the study of unanesthetized animals, where a monaural acoustic stimulation is significantly easier to implement. Second, the ipsilaterally evoked effects are almost always larger than the contralateral effects (e.g., Fig. 2).

The present results also clearly point out the dangers of using a DPOAE-based assay to measure OC reflex strength, given that the magnitudes of the effects can vary over such a large range with primary level ratio (Figs. 4 and 5). Note that this caveat applies equally to the conventional contralateral sound approach to measurement of the contralateral reflex as to the post-onset adaptation approach used here. Our experiments clearly demonstrate that, when using such assays, a range of primary level ratios must be evaluated. With any single level ratio, the OC effect observed can vary by tens of dB, and even change its sign. Nevertheless, the reproducibility of effects across a population of normal guinea pigs suggests that the assay can be reliable (Fig. 5) so long as a reasonably large matrix of level-ratio combinations is assessed.

The rapid onset effects easily recorded in these rodent ears may be more difficult to study in human ears, given their smaller OAE responses and generally higher background noise levels of awake subjects. Strategies that compare steady-state DPOAE amplitudes to primaries with short vs. long interstimulus intervals (i.e., activated vs. inactivated OC neurons) may provide a means to quantify the ipsilateral effects in those ears (see Liberman et al. 1996).

Effects of OC feedback on conventional measures of DPOAE amplitude

A corollary of the idea that post-onset DPOAE adaptation is an OC-mediated effect is the postulate that efferent feedback, evoked by the primary tones themselves, can modify the steady-state amplitude of the DPOAE. Given that most conventional measures of DPOAE amplitudes, in the clinic or the laboratory, monitor only steady state, it is clear that all these measures must be shaped, at least to some extent, by the presence of an intact OC system. The present results show that this is true, even in anesthetized animals (e.g., Fig. 7).

For many primary frequencies and level ratios, these effects will be quite small, less than 5 dB (although primary level ratios in which L_2 is 5–10 dB below L_1 are popular). A previously unexplained example of this type of small, but clearcut, effect appears in earlier studies of *in vivo* pharmacology of OC effects on DPOAEs. In two separate sets of experiments (Kujawa et al. 1994, 1995), intracochlear perfusion of antagonists of the OC receptor *increased* DPOAE amplitudes (~2–4 dB) from predrug baselines. For the parameters of continuous stimulation used in those studies, ipsilateral OC effects should have resulted in a small, negative effect on steady-state amplitudes; thus, pharmacologic blockade would be expected to result in steady-state amplitude enhancement, as observed. Increases were seen for antagonists that block the nicotinic receptors on the OHCs (e.g., curare, strychnine, and bicuculline). At the same concentrations, they were not observed for muscarinic antagonists nor for consecutive perfusions of the control solution. To our knowledge, the pharmacology of the ipsilaterally responsive OC neurons has not been systematically investigated. These observations, however, suggest that the receptor mediating these effects has the same pharmacology as the receptor mediating contralateral sound effects.

According to the present results, the effects of OC feedback on conventionally measured DPOAEs can, under some circumstances, be much larger (>10 dB). In our data, such prominent changes in steady-state DPOAE amplitude always occurred in regions where there were prominent dips, or nonmonotonicities, in the amplitude-vs.-level functions (Figs. 5 and 7). After the OC section, such dips were greatly reduced or eliminated, and steady-state amplitudes were either increased or decreased, depending on primary level ratio. Although these nonmonotonicities were remarkably stereotyped for the guinea pig, presumably they

will be idiosyncratic to each species with respect to the stimulus parameters at which they appear prominent. However, the overall principle, i.e., that at least some of the prominent dips in DPOAE amplitude-vs.-level functions are OC-mediated, may be generally applicable. Such effects may be important in shaping the averaged, steady-state DPOAEs routinely recorded from human ears in clinical settings. The presence of hearing loss, of course, would be expected to alter these effects; both because the effects are exquisitely sensitive to stimulus level and frequency and because the direct targets of the MOC efferents, the OHCs, frequently are compromised in peripheral hearing loss.

ACKNOWLEDGMENTS

The authors thank Chris Brown, John Guinan, and Chris Shera for their comments on an earlier version of this manuscript. Research was supported by grants from the NIDCD: RO1 DC00188 and F32 DC00180.

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