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Effects of Anesthesia on Efferent-Mediated Adaptation of the DPOAE

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ABSTRACT

Distortion product otoacoustic emissions (DPOAE) adapt after primary tone onset, with an ~100 ms time constant, due to feedback effects of medial olivocochlear (MOC) activity elicited by the primary tones. We tracked DPOAE postonset adaptation as a metric of MOC reflex strength, before during and after induction of anesthesia in guinea pigs. Reflex strength was significantly diminished by the barbiturate/neuroleptic anesthesia most commonly used in this species. The MOC reflex recovered more slowly than toe-pinch or startle reflexes, correlating better with recovery of general mobility. When individual anesthetic agents were assessed, the barbiturate (pentobarbital) significantly diminished MOC reflex strength, whereas fentanyl or droperidol did not. These results suggest that previous studies using anesthetized preparations may have underestimated the magnitude of sound-evoked responses in the OC pathway.

Keywords: olivocochlear reflex, otoacoustic emission, sodium pentobarbital, superior olivary complex, cochlea

INTRODUCTION

The olivocochlear (OC) pathway of efferent neurons allows central control of cochlear processing (reviewed by Guinan 1996; Warr 1992). Medial olivocochlear (MOC) neurons, one component of the pathway, respond to sound and form the efferent limb of an MOC reflex. Single-unit recordings of MOC neurons have revealed a number of their physiological characteristics (Robertson and Gummer 1985; Gummer et al. 1988; Liberman and Brown 1986; Liberman 1988; Brown 1989; Brown et al. 1998a,b): They respond with regular interspike intervals to pure tones and noise, have moderately long latencies of 5–50 ms, and are sharply tuned, with best thresholds close to that of primary afferents. MOC discharge generally increases monotonically with sound level; however, rates above ~80 spikes/s are uncommon. Using monaural sound, some MOC neurons respond only to ipsilateral sound, others only to contralateral sound, and a small minority to sound in either ear. Almost all MOC neurons, however, are binaural in that the nonresponsive ear can facilitate the response to the excitatory ear. Lastly, MOC neurons show significantly less adaptation than primary afferents of the auditory nerve (Brown 2001).

These studies of MOC neurophysiology have used anesthetized animals. Anesthesia, however, is known to depress reflex pathways and thus may alter MOC activity. It is important to understand how much, and in what ways, anesthesia alters the MOC reflex, in part so that we can interpret physiological results from anesthetized preparations. In the present study, a barbiturate and neuroleptic anesthesia was tested (Evans 1979) that has often been used in physiolog-

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ical studies of the MOC system in the guinea pig. To evaluate the effects of this anesthesia on the MOC system, we exploited the recently developed monaural test of MOC reflex strength that is based on postonset adaptation of the distortion product otoacoustic emissions (DPOAEs). In most past reports, this test has been applied to anesthetized animals (Liberman et al. 1996; Sun and Kim 1999; Kujawa and Liberman 2001). We found that, with training, this test could also be applied to awake guinea pigs with minimal restraint (Maison and Liberman 2000). Thus, the MOC reflex strength could be monitored before and during the onset and duration of anesthesia.

Previous studies suggest that DPOAE postonset adaptation is mediated by the MOC system, since sectioning the OC bundle virtually eliminates it (Liberman et al. 1996; Kujawa and Liberman 2001). The effect is thought to arise because the primary tones evoke activity in MOC neurons that project to cochlear outer hair cells, which, in turn, are involved in amplifying the DPOAE. The time constant of DPOAE postonset adaptation (100–200 ms) is consistent with other sound and shock-evoked peripheral OC effects. These peripheral time constants are thought to be dominated by postsynaptic events at the outer hair cell. In our monaural DPOAE paradigm, as in others that use sound to activate the MOC neurons (Warren and Liberman 1989; Veuillet et al. 1991), the peripheral effects are maximal at moderate and high sound levels, presumably because a robust response is required from MOC neurons. Many previous studies have used contralateral sound to activate the MOC neurons (Liberman 1989; Mott et al. 1989; Puel and Rebillard 1990; Kalluri and Shera 2001), but in awake animals it is difficult to position both an ipsilateral and a contralateral sound source. Our reflex assay used a single ipsilateral sound source and measured the adaptation produced by the ipsilateral primary tones. Given that MOC neurons responding to ipsilateral sound represent the largest subpopulation of the pathway (Robertson and Gummer 1985; Liberman and Brown 1986; Brown et al. 1998a), we are measuring the largest portion of the MOC reflex.

METHODS

All procedures were conducted in accordance with guidelines of the National Institutes of Health and were approved by the Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary. Experiments were conducted within a sound-shielded chamber (Ver et al. 1975). Adult guinea pigs (298–648 g) were selected for this study if they had prominent postonset adaptation of the DPOAE. Other animals with small adaptations were not included (Maison and Liberman 2000). A total of 13 guinea pigs were used: two for the tests of gentamicin (e.g., Fig. 2G), seven for the combined effects of barbiturate/neuroleptic anesthesia (e.g., Figs. 3 and 7), and four for investigations of individual anesthetic agents (Fig. 5).

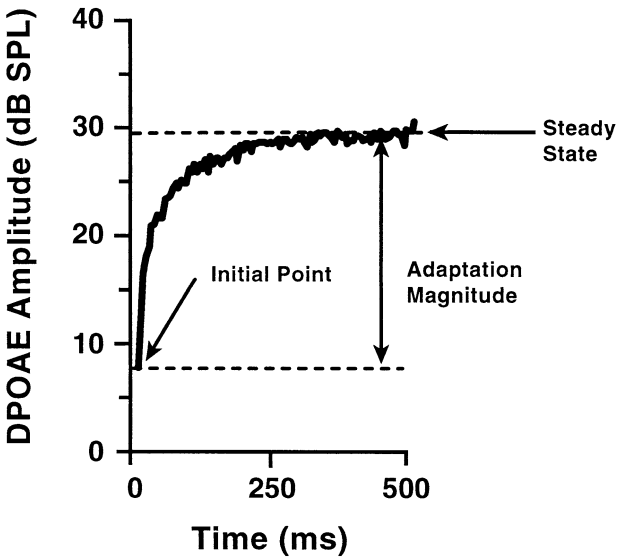


FIG. 1. Postonset adaptation of the $2f_1 - f_2$ DPOAE after onset of primary tones in an awake guinea pig (f_1 =8.3 kHz at 78 dB SPL, f_2 = 10 kHz at 70 dB SPL). Adaptation magnitude is defined as the difference between the initial point and the steady state, the latter measured as the average of the last 10 samples (final 102.4 ms). For this trace, adaptation magnitude was −21.9 dB.

DPOAEs were measured with an Etymotic Research (ER) 10C acoustic system (Etymotic Research, ElkGrove Village, IL). Two primary tones (f_1 = 8.3 kHz and f_2 = 10 kHz) were generated digitally (20 μs sampling) using a D–A board (AO-6, National Instruments, Austin, TX) in a Macintosh computer (Apple, Cupertino, CA) operating under Lab VIEW control. Only ipsilateral sounds were presented. Ear-canal sound pressure was measured with a low-noise microphone in the ER10C probe. The microphone output was amplified and digitized by an A–D board (A-2000, National Instruments). The microphone was calibrated in a coupler with a 1/4-in. condenser microphone (Brüel & Kjaer, Langen, Germany). The tip of the acoustic probe was held just outside the ear canal, just touching the tragus. Primary tone duration was 512 ms, and there was a 2.8-s interval between stimulus presentations. The digitized microphone output was broken into contiguous samples of 10.24 ms. For each sample, a fast Forier transform (FFT) was computed and the amplitude at $2f_1 - f_2$ was extracted and plotted versus postonset time (e.g., Fig. 1).

Primary levels were varied in 1-dB steps: six or more steps of L_1 within the range 74–84 dB SPL, and

at least 14 steps of L_2 beginning at 60 dB and increasing to L_1 (with $L_1 \leq L_2$). One complete ''matrix'' of all primary level combinations (e.g., Fig. 2) required at least 20 minutes to obtain. One matrix was obtained for the awake state and for each of the following states, in each of which the guinea pig was sedated and generally immobile: (1) anesthetized [toe-pinch reflex absent (−), startle reflex absent], (2) initial recovery from anesthesia [toe-pinch reflex present (+), startle reflex absent], (3) later recovery from anesthesia (toe-pinch reflex present, startle reflex present). The toe-pinch reflex stimulus was a fingernail pinch to the hind paw, and the startle reflex stimulus was an auditory click of about 80 dB SPL. For tests in the awake condition, the guinea pig was gently held by an assistant. In the anesthetized condition, the animal was placed ventral side down on a heating pad with the same head orientation as when awake. Rectal temperature was controlled to 37°C after the anesthetics were administered. Anesthesia was a barbiturate/neuroleptic combination (Evans 1979) consisting of sodium pentobarbital (35 mg/kg IP) followed 15 minutes later by droperidol (7.5 mg/kg IM) and fentanyl (0.15 mg/kg IM). In four guinea pigs, only one of the agents was used at the same dosages. In two awake guinea pigs, gentamicin was administered at 200 mg/kg IM in order to achieve a pharmacological block of the MOC reflex (Smith et al. 1994; Avan et al. 1996).

In three of the guinea pigs, myringotomies were performed using a No. 27 gauge needle. In the first guinea pig, the myringotomy was performed the day before the anesthesia test while the animal was sedated by sodium pentobarbital (35 mg/kg IP) and local anesthetic. In the other two guinea pigs, the myringotomies were performed during the anesthesia tests after the induction of the barbiturate/neuroleptic anesthesia.

RESULTS

DPOAE adaptations as a metric of OC reflex strength

Previous work in anesthetized cats and guinea pigs has shown that the DPOAE at $2f_1–f_2$ displays prominent postonset adaptation, if the DPOAE magnitude is sampled with fine time resolution (~10 ms per point) after sudden onset of the primary tones. This phenomenon (illustrated in Fig. 1) likely arises from the peripheral effects of activation of the olivocochlear (OC) pathway evoked by the primary tones themselves. The time course of postonset DPOAE adaptation is roughly exponential, with an onset time constant of ~100 ms, i.e., consistent with that observed for the peripheral effects of the OC system whether evoked by electric shocks or by contralateral sound (Wiederhold and Kiang 1970; Warren and Liberman 1989). The strongest evidence that postonset adaptation is OC mediated comes from the observation that it almost completely disappears with surgical interruption of the OC bundle (Liberman et al. 1996; Kujawa and Liberman 2001).

The magnitude of postonset adaptation is defined as the difference between the initial point and the steady state (mean of last 10 points). Previous work in anesthetized guinea pigs showed that adaptation magnitude and sign were extremely sensitive to primary level ratio (e.g., Fig. 2A vs. B). An example of this relation, in an awake animal from the present study, is illustrated by one row of the matrix in Figure 2E. In this display, L_1 and L_2 (the levels of the two primary tones f_1 and f_2) are the two axes of the matrix, and DPOAE magnitude and sign are coded via saturation and hue, respectively. With L_1 fixed at 79 dB SPL and L_2 stepped from 60 to 77 dB, adaptation magnitude increased (with increasing L_2) from small to large negative values (Fig. 2A, L_2 = 70 dB), then abruptly flipped sign to positive values (Fig. 2B, L_2 = 74 dB), and then slowly decreased as L_2 approached L_1. This abrupt sign change was always associated with a local minimum in steady-state DPOAE magnitudes (see below).

Adaptation's sign ambiguity is not inconsistent with an OC-mediated effect, given that shock-evoked OC activation has been observed to cause both increases and decreases in steady-state DPOAE amplitude (Siegel and Kim 1982). The sign ambiguity is not surprising given that ear-canal DPOAEs may reflect interactions between two intracochlear sources (one near the f_2 place and a second near the $2f_1–f_2$ place) which may have different phases (Kim et al. 1980; Brown 1987; Whitehead et al. 1992a,b; Kalluri and Shera 2001). Since postonset adaptation is largest in L_1/L_2 regions where DPOAE amplitudes show a local minimum, there is probably an association with a cancellation process involving these different components. In such a cancellation region, small changes in one component could produce large changes in the overall DPOAE.

The choice of stimulus parameters in the present study (f_1 = 8.3 kHz, L_1 from 74 to 84 dB; f_2 = 10 kHz, L_2 from 60 to 84 dB) was guided by previous work in anesthetized guinea pigs. In that preparation, DPOAE adaptation magnitude was maximal for f_2 near 8–10 kHz, consistent with recordings from single OC fibers which show maximal sound-evoked activity among fibers with characteristic frequencies (CFs) near 8–10 kHz. DPOAE adaptation magnitude was also maximal for high primary levels (65–80 dB SPL), presumably reflecting the optimal tradeoff between two opposing trends i.e., OC effects on cochlear

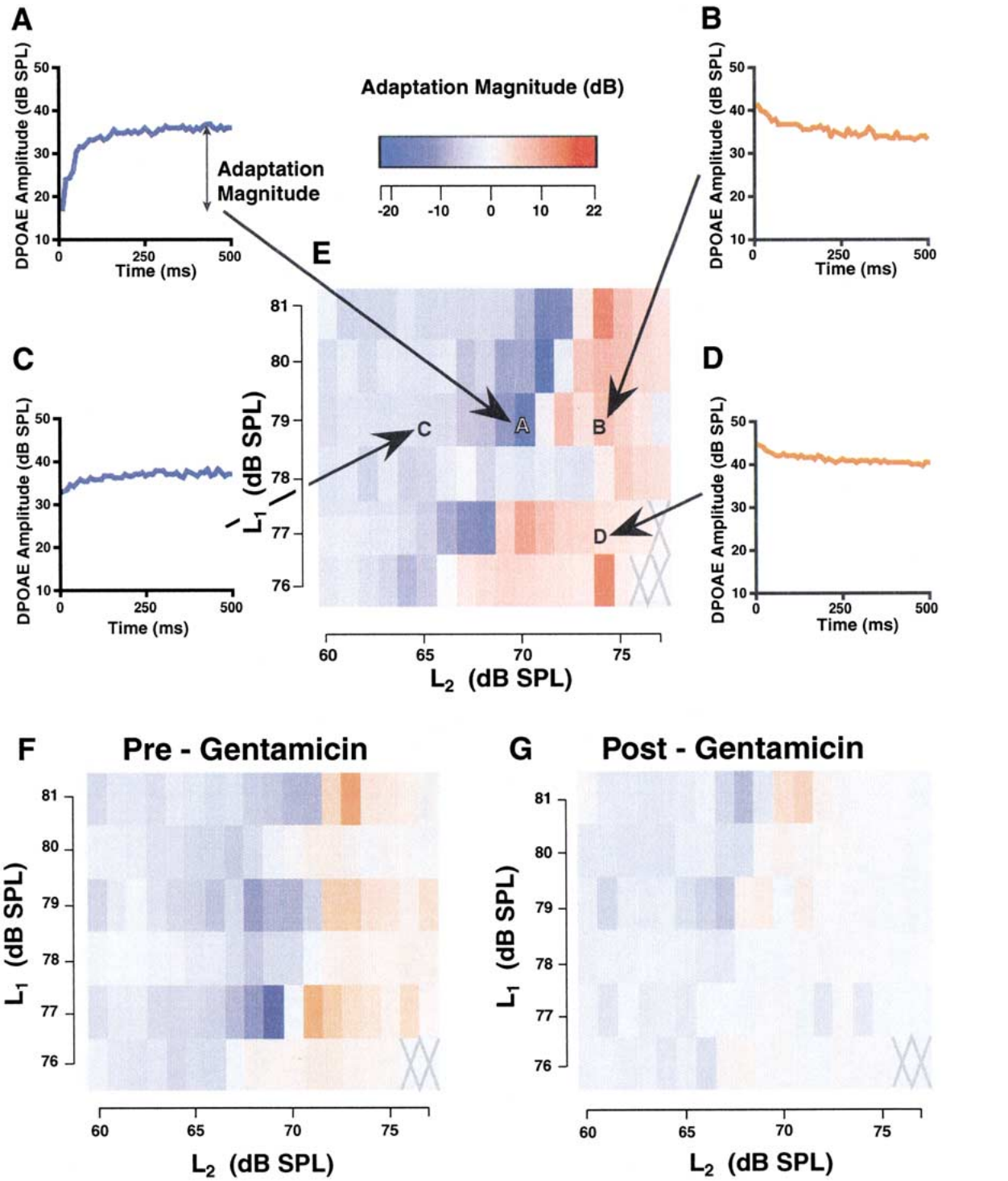


FIG. 2. DPOAE data from awake guinea pigs illustrating magnitude and sign of postonset adaptation at a matrix of 6 × 18 primary-tone levels (top panels). **A–D**. Individual traces of postonset adaptation at four different level combinations. Adaptation magnitude is indicated in **A**. **E.** Adaptation magnitude plotted for each L_1/L_2 combination, color-coded with purple and orange hues for positive and negative signs, respectively, and adaptation magnitude coded as degree of saturation (see scale). L_1 and L_1 refer to the sound pressure levels of the two primary tones. **E, F.** For another awake guinea pig, two matrices demonstrating that gentamicin decreases postonset adaptation. Panels show matrices of adaptation magnitudes for the following states: (**F**) before, and (**G**) 4 hours after administration of gentamicin (200 mg/kg IM). X's show cells for which there are no data.

responses, including DPOAEs, tend to be largest at low-stimulus SPLs, yet sound-evoked OC activity will grow monotonically with increasing SPL of the evoking primaries.

Although in previous studies the overall relation between primary levels and DPOAE magnitude/sign behavior was quite stereotyped across animals, the precise level ratios eliciting the largest postonset adaptations differed slightly (Kujawa and Liberman 2001). In recognition of this variation, our metric of OC reflex strength in the present study included a large matrix (>150 L_1L_2 combinations) to insure that anesthesia-induced changes in the primary levels eliciting large OC effects were not misinterpreted as a loss of reflex effects. An overall measure of reflex strength was defined as follows: for each L_1 the absolute values of the largest positive and largest negative adaptation magnitudes were summed and these sums for the six contiguous L_1 values in the matrix were averaged. For the matrix of Figure 2E, the reflex strength was 23.9 dB.

Although DPOAE postonset adaptation in anesthetized animals has been shown to be clearly tied to the OC system by acute cuts of the bundle (Liberman et al. 1996; Kujawa and Liberman 2001), in the present study we used intramuscular injection of gentamicin to demonstrate that the phenomenon in unanesthetized animals is also OC mediated. Gentamicin has been shown to block the MOC system without ototoxic effects when given as a single injection at a dose of 200 mg/kg (Smith et al. 1994; Avan et al. 1996), although this block may not be complete (Smith et al. 2000). In two awake guinea pigs, DPOAE adaptation was measured before and after gentamicin injection. Results from one experiment are illustrated in Figure 2F,G. The large adaptations seen pregentamicin (Fig. 2F) were considerably decreased 4h after gentamicin (Fig. 2G). Both negative (purple) and positive (orange) adaptations were decreased. Reflex strength before gentamicin was 17.8 dB (Fig. 2F) and after gentamicin was 8.4 dB (Fig. 2G). A similar pattern was seen in the other animal tested.

Anesthesia-related changes in OC reflex strength

Present data tracking DPOAE postonset adaptation before and during induction of anesthesia show that OC reflex strength was clearly decreased by pentobarbital/droperidol/fentanyl anesthesia. Data from two guinea pigs are shown in Figure 3: one with a large depression (Fig. 3A–D), and another in which anesthesia had a smaller effect (Fig. 3E–H). In the awake condition (Fig. 3A,E), postonset adaptation was large, as indicated by the dark colors. In the most deeply anesthetized state (Fig. 3B,F), ~1h after anesthetic injection, both toe-pinch and startle reflexes disappeared [pinch(−)/startle(−)] and the animal was immobile. In this state, both positive and negative adaptations were reduced in magnitude and MOC reflex strength was maximally depressed. Reflex strengths, measured for the six contiguous L_1 values showing the largest adaptations, were 19.2 dB (Fig. 3A) and 23.9 dB (Fig. 3E) when awake and 8.9 dB (Fig. 3B) and 16.0 dB (Fig. 3F) when deeply anesthetized. After the deepest state of anesthesia, first the toe-pinch and then the startle reflex returned over the next several hours, although the animal still appeared to be asleep. For the data in Figure 3, the toe-pinch(+)/startle(−) state began at ~3h and the toe-pinch(+)/startle(+) state began at ~4.5 h after anesthetic injection. The matrices for these states (Fig. 3C,D and G,H) demonstrate partial recovery of reflex strength. For guinea pig 1 (left column), the recovery began during the toe-pinch(+)/startle(−) state, but for guinea pig 2 (right column), the reflex strength became somewhat lower during this state and did not begin to recover until the toe-pinch(+)/startle(+) state. Note that for guinea pig 1, during initial recovery (Fig. 3C) a region of large DPOAE adaptation appeared first at the highest primary levels (upper right of matrix) and later shifted to lower levels as in the original awake condition. (Our OC reflex metric tracks the maximum effect by selecting the 6 contiguous L_1 levels with largest postonset adaptations). In contrast, the pattern of recovery for guinea pig 2, as for most other animals not illustrated, did not show obvious shifts in L_1/L_2 values at which maximal adaptations were seen.

Average reflex strength in the different anesthetic states for all six guinea pigs in the present study is summarized in Figure 4. On average, in the most deeply anesthetized condition, the reflex strength metric was reduced approximately by half, from an average of 19.8 dB in the awake condition to 11.4 dB in the toe-pinch(−)/startle(−) condition. In fact, there were statistically significant differences between the reflex strengths in the awake condition versus all anesthetized states (Fig. 4). Thus, even after toe-pinch and startle reflexes had reappeared, OC reflex strength showed only slight recovery. In this respect the MOC reflex correlated better with sedation level because in this lightest state of anesthesia the animal was still immobile and lacked a righting reflex. Two of the six guinea pigs were measured the day following anesthetization. At this point, the animals were fully mobile and both showed almost complete recovery in MOC reflex strength. For example, one such measurement (left column of Fig. 3) showed a preanesthesia reflex strength of 19.2 dB, recovering to 17.9 dB by the next day. In all these assays of the effects of anesthesia, both positive and negative adaptations were reduced about the same amounts, with positive

adaptations falling on average to 51.3% of their awake values and negative adaptations falling on average to 64.4% of their awake values, a difference that was not statistically significant.

All the data in Figures 3 and 4 were for an anesthetic protocol commonly used for physiological study in the guinea pig, i.e., three anesthetic agents (pentobarbital, droperidol, and fentanyl) given together. In a few experiments, we evaluated the effects of each agent independently. There was a clear reduction in MOC reflex strength when sodium pentobarbital was given alone in two guinea pigs, one in which the tympanic membrane was intact (Fig. 5A) and one in which a myringotomy had been performed on the previous day to control for any changes in middle-ear pressure (Fig. 5B). In contrast, there was no change in reflex strength when droperidol or fentanyl were given alone, each tested in a single guinea pig (Fig. 5C,D). Fentanyl (0.15 mg/kg) alone eliminated the toe-pinch reflex; however, it did not sedate the animal enough to render it immobile, nor did it change the MOC reflex. Conversely, pentobarbital alone did not eliminate the toe-pinch reflex at the dose given (35 mg/kg), but it was the only agent that produced sedation and decreased MOC reflex strength. Once again, MOC reflex strength is better correlated with sedation level than with the toe-pinch and startle reflexes.

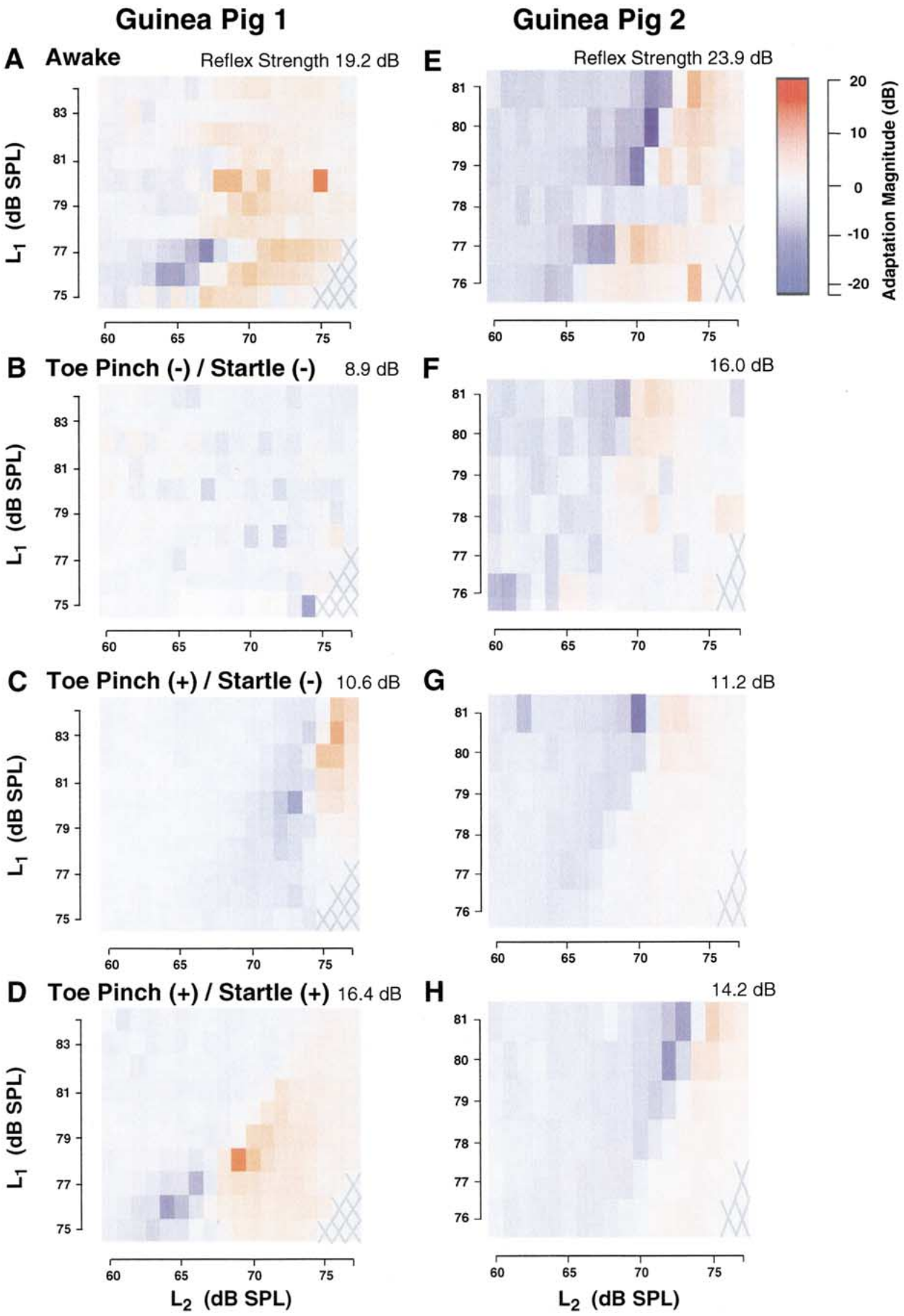
To control for anesthesia-induced changes in middle-ear pressure, which, by decreasing middle-ear transmission, could reduce effective stimulus levels and thus the degree of OC activation, two guinea pigs with myringotomies were tested. One was tested with the full anesthetic combination, and the myringotomy was performed just after anesthesia using a No.27 needle. This guinea pig showed the same type of reflex strength decline as the others (reflex strength 13.9 dB when awake and 6.4 dB after anesthesia and myringotomy). The second guinea pig had a myringotomy performed on the day prior to the anesthesia test. In this animal, which was administered pentobarbital alone, the reflex strength decline was also observed (Fig. 5B). These results suggest that the anesthesia-induced changes in postonset adaptation do not arise from changes in middle-ear pressure.

DPOAE amplitude

In addition to reducing DPOAE adaptation, anesthesia also affected the DPOAE amplitude, both the initial point after primary-tone onset and the steady-state amplitude (defined in Fig. 1). We concentrate on the steady state since steady-state amplitude changes are known to occur after section of the OC bundle (Liberman et al. 1996; Kujawa and Liberman 2001).

The effect of pentobarbital/droperidol/fentanyl anesthesia on steady-state DPOAE amplitude is illustrated in Figure 6 for the same cases illustrated in Figure 3, but now using a matrix display with amplitude coded by gray-scale value. In the awake condition (Fig. 6A,E), the amplitude generally grew (lighter shading) with increasing L_1 or L_2. However, there are clear local minima (dark shading) superimposed on that general trend, e.g., L_1 = 75–77 and L_2 = 67–74 in Figure 6A, or L_1 = 78–81 and L_2 = 74 in Figure 6B. These minima occurred near level combinations where postonset adaptation was large (Fig. 3). Anesthesia (Fig. 6B,F) tended to reduce this nonmonotonic level dependence so that minima were less prominent (e.g., Fig. 6F), in parallel with its reductions in postonset adaptation (Fig. 3B,F). The nomonotonicities returned, sometimes more prominently than before, in the toe-pinch(+)/startle(+) state (Fig. 6D,H). A similar (but irreversible) removal of notches in DPOAE growth functions has been observed following section of the OC bundle (Kujawa and Liberman 2001).

In some animals, anesthesia caused striking transient decreases in DPOAE amplitudes, but only for primary levels below those used to elicit our metric of OC reflex strength. The time scale of this effect is too fast to be captured by the L_1/L_2 matrix approach which requires more than 20 minutes to complete. Thus, the phenomenon was examined in a few animals by repeated measure of DPOAE steady-state amplitudes (pre- and postanesthetization) at a small number of primary levels (30–80 dB SPL in 10-dB steps with $L_1 = L_2 - 10$). Results of one such test are illustrated in Figure 7. For both levels illustrated (L_1 = 50 and 80 dB), variability in DPOAE amplitude was reduced after anesthesia (presumably due to decreased animal movement and associated masking noise). Within the first 30 minutes after anesthetic injection, DPOAE amplitude underwent a fast decrease and recovery, most striking for the lower-level primaries (50 dB). The fast decrease is likely caused by pentobarbital, as it occurred in the one pentobarbital-only guinea pig where time course was studied. These rapid DPOAE amplitude changes do not appear to result from changes in middle-ear pressure, as evidenced by reproducing the phenomenon in one guinea pig in which a myringotomy was performed on the day prior to the anesthesia test. Also, in another guinea pig in which the myringotomy was performed about 1 h after anesthesia induction, the myringotomy did not alter DPOAE amplitude. The nature of these rapid and reversible changes in DPOAE amplitude for low-level primaries was not systematically studied as these changes do not affect the metric of central importance to the present report.


Guinea Pig 1
Guinea Pig 2
A Awake
Reflex Strength 19.2 dB
E
Reflex Strength 23.9 dB
B Toe Pinch (-) / Startle (-)
8.9 dB
F
16.0 dB
C Toe Pinch (+) / Startle (-)
10.6 dB
G
11.2 dB
D Toe Pinch (+) / Startle (+)
16.4 dB
H
14.2 dB
L1 (dB SPL)
L2 (dB SPL)
Adaptation Magnitude (dB)
20
10
0
-10
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75
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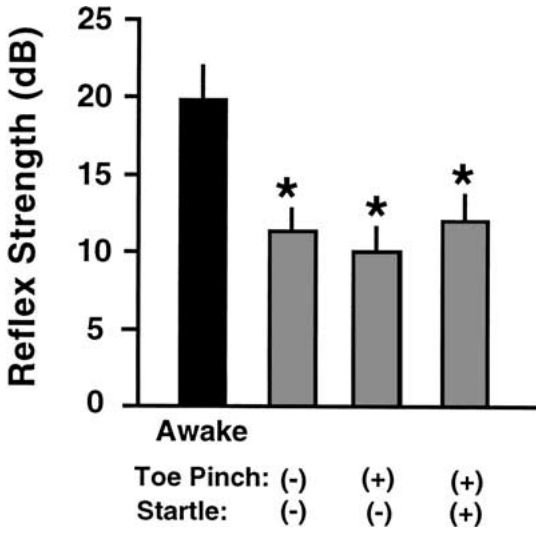


FIG. 4. Average reflex strength declines with anesthesia for six guinea pigs tested with the full barbiturate/neuroleptic anesthetic. Error bars represent standard error. Asterisks indicate that average reflex strength was significantly decreased for each anesthetic state relative to the awake state.

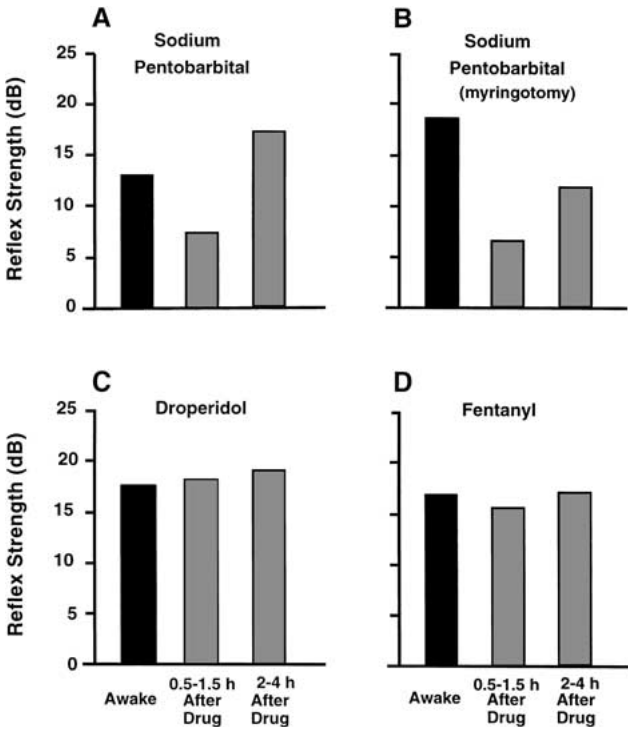


FIG. 5. Data from four guinea pigs tested with individual anesthetic agents indicate that sodium pentobarbital (**A,B**) decreases reflex strength, whereas droperidol (**C**) and fentanyl (**D**) do not. To control for changes in middle-ear pressure, one of the guinea pigs that received pentobarbital had undergone a myringotomy on the previous day (**B**). Agents were given at the same dose used in the combination barbiturate/neuroleptic anesthesia (sodium pentobarbital, 35 mg/kg IP; droperidol, 7.5 mg/kg IM; fentanyl, 0.15 mg/kg IM).

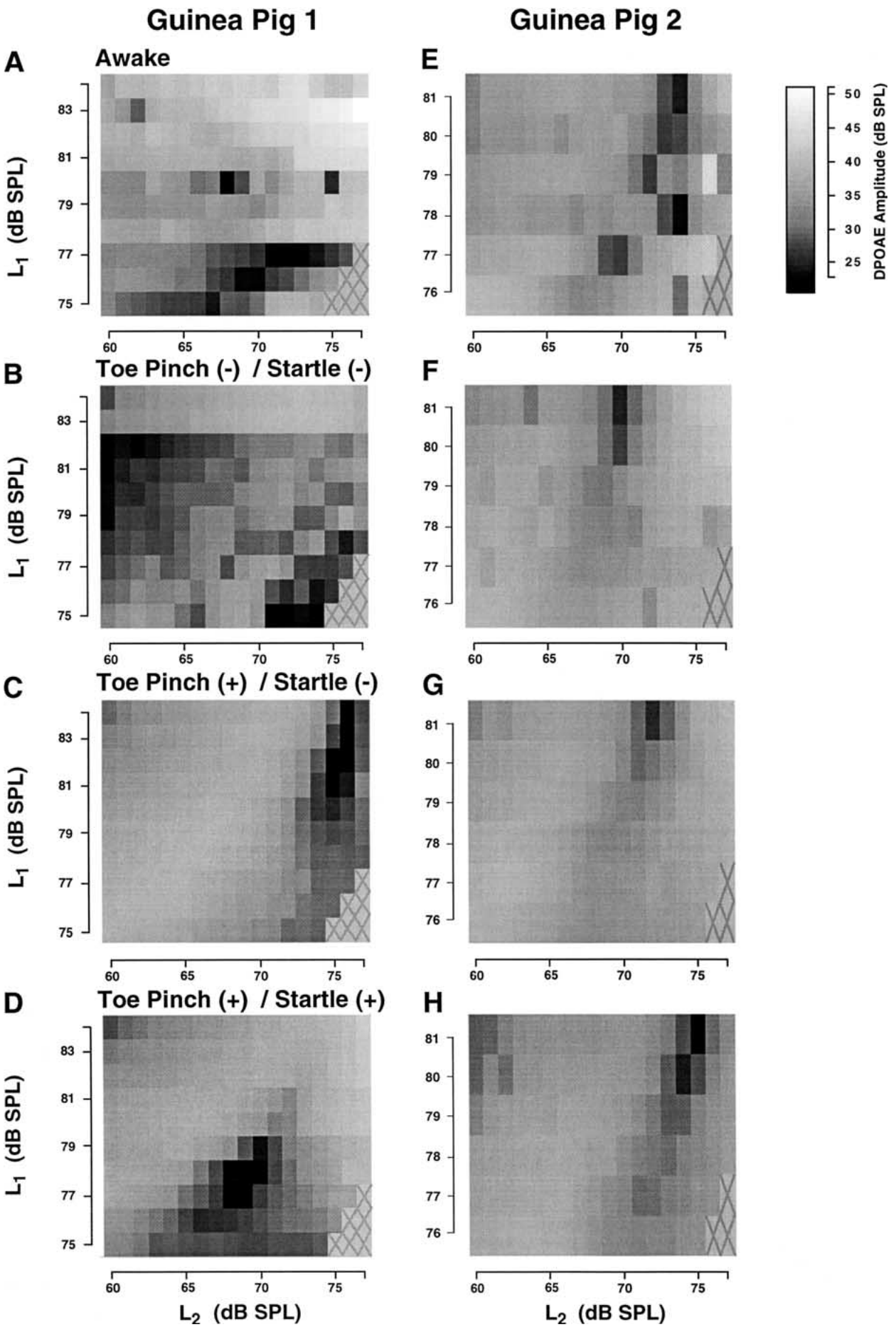
DISCUSSION

DPOAE adaptation as a metric for MOC reflex strength

Postonset adaptation of the DPOAE is considered a sensitive test for the MOC reflex for two reasons: First, the DPOAE adaptations, both positive and negative in sign, are almost completely eliminated on section of the OC bundle in anesthetized cats and guinea pigs (Liberman et al. 1996; Kujawa and Liberman 2001). Second, gentamicin, a known blocker of the MOC reflex (Smith et al. 1994; Avan et al. 1996) also greatly reduces these adaptations in guinea pig (Fig. 2). There are several arguments against a major contribution of the middle-ear muscle reflex to DPOAE postonset adaptation, at least in cats and guinea pigs. First, the phenomenon is still reliably seen in cats with section of middle-ear muscle tendons (Liberman et al. 1996) and in guinea pigs with curare-induced muscle paralysis. Second, acoustic levels used to elicit the effect are probably below those that stimulate the middle-ear muscle reflex (Carmel and Starr 1963), and the acoustic frequencies which elicit maximal effects (8–10 kHz in guinea pig) are well above those on which the middle-ear muscle reflex has large effects (Nuttall 1974; Pang and Peake 1986). Although these arguments rule out significant contribution of the middle-ear muscle reflex in our preparation, middle-ear muscles can make a large contribution to DPOAE adaptation in ketamine-anesthetized rats, at least for some frequencies and levels (Relkin et al. 2001). Clearly, the relative contributions of the two major negative feedback reflexes to the auditory periphery need to be reassessed for each species or anesthetic regimen under consideration.

For a DPOAE-based metric to be used effectively to assess anesthetic effects on the MOC reflex, we must have some confidence that the anesthetic agents are not having a direct influence on the cochlea itself. The DPOAE steady-state amplitudes provide a reasonably sensitive metric of overall cochlear condition, and, in the present study, DPOAE steady-state amplitudes away from notch regions were not greatly

FIG. 3. DPOAE adaptation matrices showing that anesthesia decreases MOC reflex strength . Panels illustrate the following states for each of two guinea pigs: (**A, E**) awake (no anesthesia), (**B, F**) anesthetized with toe-pinch reflex absent (–) and startle reflex absent (–), (**C,G**) still sedated but toe-pinch reflex preset (+) and startle reflex absentr (–) **(D, H)** still sedated with toe-pinch reflex present (+) and startle reflex present(+). Color coding as in Fig. 2. X's show cells for which there are no data. Reflex strengths (see text) are given for each panel.


Guinea Pig 1
Guinea Pig 2
A
Awake
B
Toe Pinch (-) / Startle (-)
C
Toe Pinch (+) / Startle (-)
D
Toe Pinch (+) / Startle (+)
E
F
G
H
L1 (dB SPL)
L2 (dB SPL)
83
81
79
77
75
81
80
79
78
77
76
60
65
70
75
DPOAE Amplitude (dB SPL)
50
45
40
35
30
25

affected by anesthesia, at least not the amplitudes measured in response to primaries in the 60–80-dB range used to elicit postonset adaptations. Although we did note a transient decrease in the DPOAE evoked by lower-level primaries immediately after barbiturate injection (Fig. 7), that decrease appeared to recover within 20–30 minutes, and clearcut depression of DPOAE postonset adaptation persisted for much longer than 30 minutes. Nevertheless, it is interesting to consider the mechanism of this striking transient fall in DPOAE amplitudes. By introducing small perforations in the tympanic membrane, we showed that this effect cannot be explained by changes in static middle-ear pressure. Viable alternative explanations include (1) direct effect on outer hair cells, (2) transient decreases in cochlear blood flow, or (3) a transient rise in spontaneous activity in MOC neurons.

DPOAE adaptation reduction by anesthesia and implications for the MOC reflex

Measurement of DPOAE postonset adaptation is easily applicable to the awake preparation, although, with ipsilateral stimulation, only the ipsilateral MOC reflex can be tested. The presence of a small amount of residual adaptation following MOC section in both cats and guinea pigs shows that there are other sources of DPOAE adaptation within the cochlea and suggests that, in the present study, this metric will tend to underestimate the extent to which the reflex has been attenuated by our anesthetic manipulations.

Present results show that MOC reflex strength declines with barbiturate anesthesia (Fig. 4), as does the acoustic startle and toe-pinch reflexes. In human subjects, both sleep (Froehlich et al. 1993) and benzodiazepines (Morand et al. 1998) decrease suppressive effects of contralateral noise on transient OAEs. Such effects apparently involve the OC reflex because they disappear after the OCB is severed by vestibular neurotomy (Giraud et al. 1995). The middle-ear muscle acoustic reflex is also greatly reduced by barbiturate anesthesia (Borg and Moller 1975). Thus, a variety of brainstem auditory reflexes are clearly state-dependent.

Anesthesia may decrease MOC reflex strength by decreasing sound-evoked responses of MOC neurons. This effect has long been suspected; in his original studies of MOC single-unit neurophysiology, Fex (1962, 1965) used decerebrate cats to avoid the effects of anesthesia. In anesthetized cats, anecdotal evidence suggested that discharge rates in MOC neurons decreased with increasing depth of anesthesia; however, the issue was not investigated systematically (Liberman and Browny 1986). Results of our study present the most direct evidence to date that anesthesia, particularly barbiturate anesthesia, significantly diminishes OC reflex strength. Although only the ipsilaterally evoked MOC reflex was assessed, previous work in anesthetized animals showed that reflex strength for ipsilateral and contralateral loops covary over a wide range of evoking stimuli as depth of anesthesia changes during an experiment (Kujawa and Liberman 2001). Thus, it is likely that the contralateral reflex loop is similarly attenuated by anesthesia. Taken together, these observations suggest that most studies of MOC neurophysiology have underestimated the sound-evoked discharge since they have used either pentobarbital/neuroleptic anesthesia (guinea pigs: Robertson and Gummer 1985; Brown 1989; Brown et al. 1998a) or diallyl barbiturate/urethane (cats: Liberman and Brown 1986; Liberman 1989). In future studies, the present approach could be used to identify anesthetic regimens with less dramatic effects on MOC reflex strength.

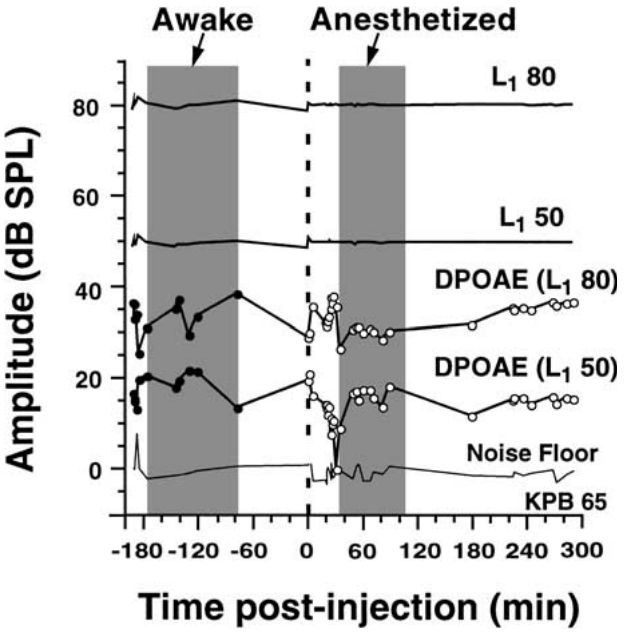


FIG. 7. Time course of DPOAE amplitude in a single guinea pig demonstrating a fast reduction and recovery at about 30 minutes after administration of anesthetic (sodium pentobarbital was given at time 0; droperidol and fentanyl were given 15 minutes later). During the construction of the matrix of DPOAE adaptations in the anesthetized condition (second shaded column), the DPOAE amplitude was stable and similar to the awake condition (first shaded column). DPOAE amplitudes are steady-state values for two different L_1/L_2 combinations: 80/70 and 50/40 dB SPL. Primary levels and noise floor are also plotted.

FIG. 6. Matrices demonstrating the effects of anesthesia on the steady-state DPOAE amplitude for the same data set shown in Fig. 3. DPOAE amplitude is represented by coding such that low amplitudes are black, medium amplitudes are gray, and high amplitudes are white (see scale). X's show cells for which there are no data.

Anesthesia may depress MOC neurons directly and/or may indericlty by acting on elements of the reflex pathway or *alter* descending projections that modulate the reflex. Elements of the MOC reflex arc have not been completely defined, but the afferent limb consists of auditory nerve projections to the cochlear nucleus, where the next stage likely involves the posteroventral subdivision (Thompson and Thompson 1991; de Venecia et al. 2001) and the anteroventral subdivision (Robertson and Winter 1988; Ye et al. 2000), which project directly to the cells of origin of the MOC system. Effects of anesthesia on the posteroventral subdivision neurons have not been well studied (Godfrey et al. 1975). However, cats with supracollicular decerebration, which should not disturb the MOC reflex pathway from cochlear nucleus to superior olivary complex and back, do not show larger sound-evoked MOC effects than cats under barbiturate anesthesia (Kawase and Liberman 1993). This decerebration would interrupt the descending projections that MOC neurons receive from auditory cortex (Mulders and Robertson 2000a) and possibly those from inferior colliculus (Faye–Lund 1986; Thompson and Thompson 1993; Vetter et al. 1993). Both of these centers send a projection to MOC neurons that has an excitatory influence (Mulders and Robertson 2000b; Khalfa et al. 2001), and both centers have responses that can be greatly altered by anesthesia (Kuwada et al. 1989; Kiang et al. 1961). Thus, if these excitatory descending influences are the major target of anesthetic effects in the present study, this would be consistent with the observation that sound-evoked MOC effects do not appear larger in decerebrate versus barbiturate-anesthetized cats.

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REFERENCES

AVAN P, ERRE J-P, LIMA DA COSTA D, ARAN J-M, POPELÁR J. The efferent-mediated suppression of otoacoustic emissions in awake guinea pigs and its reversible blockage by gentamicin. Exp. Brain Res. 109:9–16, 1996.

BORG E, MOLLER AR. Effect of central depressants on the acoustic middle ear reflex in rabbit. Acta Physiol. Scand. 94:327–338, 1975.

BROWN AM. Acoustic distortion from rodent ears: A comparison of responses from rats, guinea pigs and gerbils. Hear. Res. 31:25–38, 1987.

BROWN MC. Morphology and response properties of single olivocochlear fibers in the guinea pig. Hear. Res. 40:93–110, 1989.

BROWN MC. Response adaptation of medial olivocochlear neurons is minimal. J. Neurophysiol. 86:2381–2392, 2001.

BROWN MC, KUJAWA SG, DUCA ML. Single olivocochlear neurons in the guinea pig: I. Binaural facilitation of responses to high-level noise. J. Neurophysiol. 79:3077–3087, 1998a.

BROWN MC, KUJAWA SG, LIBERMAN MC. Single olivocochlear neurons in the guinea pig: II. Response plasticity due to noise conditioning. J. Neurophysiol. 79:3088–3097, 1998b.

CARMEL PW, STARR A. Acoustic and non-acoustic factors modifying middle-ear muscle activity in waking cats. J. Neurophysiol. 26:598–616, 1963.

DE VENECIA RK, LIBERMAN MC, GUINAN JJ JR, BROWN MC. Effects of kainate lesions in different cochlear nucleus regions on the MOC reflex. Abstr. Assoc. Res. Otolaryngol. 23:46, 2001.

EVANS EF. Neuroleptanesthesia for the guinea pig. Arch. Otolaryngol. 105:185–186, 1979.

FAYE–LUND H. Projection from the inferior colliculus to the superior olivary complex in the albino rat. Anat. Embryol. 175:35–52, 1986.

FEX J. Auditory activity in centrifugal and centripetal cochlear fibers in cat. Acta Physiol. Scand. 55(Suppl 189):1–68, 1962.

FEX J. Auditory activity in uncrossed centrifugal cochlear fibers in cat. Acta Physiol. Scand. 64:43–57, 1965.

FROEHLICH P, COLLET L, VALATX JL, MORGON A. Sleep and active cochlear micromechanical properties in human subjects. Hear. Res. 66:1–7, 1993.

GIRAUD AL, COLLET L, CHERY-CROZE S, MAGNAN J, CHAYS A. Evidence of a medial olivocochlear involvement in contralateral suppression of otoacoustic emissions in humans. Brain Res. 705: 15–23, 1995.

GODFREY DA, KIANG NYS, NORRIS BE. Single unit activity in the posteroventral cochlear nucleus of the cat. J. Comp. Neurol. 162:247–268, 1975.

GUINAN JJ JR. The physiology of olivocochlear efferents. In: Dallos P, Popper AN, and Fay RR (eds) The Cochlea. New York, Springer–Verlag, pp 435–502, 1996.

GUMMER M, YATES GK, JOHNSTONE BM. Modulation transfer functions of efferent neurones in the guinea pig cochlea. Hear. Res. 36:41–52, 1988.

KALLURI R, SHERA CA. Distortion-product source unmasking: A test of the two-mechanism model for DPOAE generation. J. Acoust. Soc. Am. 109:622–637, 2001.

KAWASE T, LIBERMAN MC. Antimasking effects of the olivocochlear reflex. I. Enhancement of compound action potentials to masked tones. J. Neurophysiol. 70:2519–2532, 1993.

KHALFA S, BOUGEARD R, MORAND N, VEUILLET E, ISNARD J, GUENOT M, RYVLIN P, FISCHER C, COLLET L. Evidence of peripheral auditory activity modulation by the auditory cortex in humans. Neuroscience 104:347–358, 2001.

KIANG NYS, NEAME JH, CLARK LF. Evoked cortical activity from auditory cortex in anesthetized and unanesthetized cats. Science 133:1927–1928, 1961.

KIM DO, MOLNAR CE, MATTHEWS JW. Cochlear mechanics: Nonlinear behavior in two-tone responses as reflected in cochlear-nerve responses and in ear-canal sound pressure. J. Acoust. Soc. Am. 67:1704–1721, 1980.

KUJAWA S, LIBERMAN MC. Effects of olivocochlear feedback on distortion product otoacoustic emissions in guinea pig. J. Assoc. Res. Otolaryngol. 2:268–278, 2001.

KUWADA S, BATRA R, STANFORD TR. Monaural and binaural response properties of neurons in the inferior colliculus of the rabbit:

Effects of sodium pentobarbital. J. Neurophysiol. 61:269–282, 1989.

LIBERMAN MC. Response properties of cochlear efferent neurons: Monaural vs. binaural stimulation and the effects of noise. J. Neurophysiol. 60:1779–1798, 1988.

LIBERMAN MC. Rapid assessment of sound-evoked olivocochlear feedback: Suppression of compound action potentials by contralateral sound. Hear. Res. 38:47–56, 1989.

LIBERMAN MC, BROWN MC. Physiology and anatomy of single olivocochlear neurons in the cat. Hear. Res.24:17–36, 1986.

LIBERMAN MC, PURIA S, GUINAN JJ JR. The ipsilaterally evoked olivocochlear reflex causes rapid adaptation of the $2f_1 - f_2$ distortion product otoacoustic emission. J. Acoust. Soc. Am. 99:3572–3584, 1996.

MAISON SF, LIBERMAN MC. Predicting vulnerability to acoustic injury with a noninvasive assay of olivocochlear reflex strength. J. Neurosci. 20:4701–4707, 2000.

MORAND N, VEUILLET E, GAGNIEU MC, LEMOINE P, COLLET L. Benzodiazepines alter cochleocochlear loop in humans. Hear. Res. 121:71–75, 1998.

MOTT JB, NORTON SJ, NEELY ST, WARR WB. Changes in spontaneous otoacoustic emissions produced by acoustic stimulation of the contralateral ear. Hear. Res. 38:229–242, 1989.

MULDERS WHAM, ROBERTSON D. Evidence for direct cortical innervation of medial olivocochlear neurones in rats. Hear. Res. 144:65–72, 2000a.

MULDER WHAM, ROBERTSON D. Effects on cochlear responses of activation of descending pathways from the inferior colliculus. Hear. Res. 149:11–23, 2000b.

NUTTALL AL. Measurements of the guinea-pig middle-ear transfer characteristic. J. Acoust. Soc. Am. 56:1231–1238, 1974.

PANG XD, PEAKE WT. How do contractions of the stapedius muscle alter the acoustic properties of the ear? In: Allen JB, Hubbard A, Neely SI, and Tubis A (eds) Peripheral Auditory Mechanisms. New York, Springer–Verlag, pp 36–43, 1986.

PUEL J-L, REBILLARD G. Effect of contralateral sound stimulation on the distortion product $2F_1 - F_2$. Evidence that the medial efferent system is involved. J. Acoust. Soc. Am. 87:1630–1635, 1990.

RELKIN EM, STERNS A, SCHIPPER J, AZEREDO W, WOODS CI. The role of the middle-ear reflex in determining the time course of DPOAEs in the rat. Abstr. Assoc. Res. Otolaryngol. 24:6, 2001.

ROBERTSON D, GUMMER M. Physiological and morphological characterization of efferent neurons in the guinea pig cochlea. Hear. Res. 20:63–77, 1985.

ROBERTSON D, WINTER IM. Cochlear nucleus inputs to olivocochlear neurones revealed by combined anterograde and retrograde labelling in the guinea pig. Brain Res. 462:47–55, 1988.

SIEGEL JH, KIM DO. Efferent neural control of cochlear mechanics? Olivocochlear bundle stimulation affects cochlear biomechanical nonlinearity. Hear. Res. 6:171–182, 1982.

SMITH DW, ERRE J-P, ARAN J-M. Rapid, reversible elimination of medial olivocochlear efferent function following single injections of gentamicin in the guinea pig. Brain Res. 652:243–248, 1994.

SMITH DW, TURNER DA, HENSON MM. Psychophysical correlates of contralateral efferent suppression. I. The role of the medial olivocochlear system in ''central masking'' in nonhuman primates. J. Acoust. Soc. Am. 107:933–941, 2000.

SUN X-M, KIM DO. Adaptation of $2f_1 - 2f_2$ distortion product otoacoustic emission in young-adult and old CBA and C57 mice. J. Acoust. Soc. Am. 105:3399–3409, 1999.

THOMPSON AM, THOMPSON GC. Posteroventral cochlear nucleus projections to olivocochlear neurons. J. Comp. Neurol. 303: 267–285, 1991.

THOMPSON AM, THOMPSON GC. Relationship of descending inferior colliculus projections to olivocochlear neurons. J. Comp. Neurol. 335:402–412, 1993.

VER IL, BROWN RM, KIANG NYS. Low-noise chambers for auditory research. J. Acoust. Soc. Am. 58:392–398, 1975.

VETTER DE, SALDANA E, MUGNAINI E. Input from the inferior colliculus to medial olivocochlear neurons in the rat: A double label study with PHA-L and cholera toxin. Hear. Res. 70:173–186, 1993.

VEUILLET E, COLLET L, DUCLAUX R. Effect of contralateral acoustic stimulation on active cochlear micromechanical properties in human subjects: Dependence on stimulus variables. J. Neurophysiol. 65:724–735, 1991.

WARR WB. Organization of olivocochlear efferent systems in mammals. In: Webster DB, Popper AN, and Fay RR (eds) The Mammalian Auditory Pathway: Neuroanatomy. New York, Springer–Verlag, pp 410–448, 1992.

WARREN EH III, Liberman MC. Effects of contralateral sound on auditory-nerve responses II. Dependence on stimulus variables. Hear. Res. 37:105–122, 1989.

WHITEHEAD ML, LONSBURY–MARTIN BL, MARTIN GK. Evidence for two discrete sources of $2f_1 - f_2$ distortion-product otoacoustic emission is rabbit: I. Differential dependence on stimulus parameters. J. Acoust. Soc. Am. 91:1587–1607, 1992a.

WHITEHEAD ML, LONSBURY–MARTIN BI, MARTIN GK. Evidence for two discrete sources of $2f_1 - f_2$ distortion-product otoacoustic emission is rabbit: II. Differential physiological vulnerability. J. Acoust. Soc. Am. 92:2662–2682, 1992b.

WIEDERHOLD ML, KIANG NYS. Effects of electric stimulation of the crossed olivocochlear bundle on single auditory-nerve fibers in the cat. J. Acoust. Soc. Am. 48:950–965, 1970.

YE Y, MACHADO DG, KIM DO. Projection of the marginal shell of the antero ventral cochlear nucleus to olivocochlear neurons in the cat. J. Comp. Neurol 420:127–138, 2000.