JARO 6: 48–62 (2005)
DOI: 10.1007/s10162-004-5009-2

JARO
Journal of the Association for Research in Otolaryngology

Disruption of Lateral Olivocochlear Neurons via a Dopaminergic Neurotoxin Depresses Sound-Evoked Auditory Nerve Activity

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Received: 30 January 2004; Accepted: 29 October 2004; Online publication: 2 February 2005

ABSTRACT

We applied the dopaminergic (DA) neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to the guinea pig cochlear perilymph. Immunolabeling of lateral olivocochlear (LOC) neurons using antibodies against synaptophysin was reduced after the MPTP treatment. In contrast, labeling of the medial olivocochlear innervation remained intact. As after brainstem lesions of the lateral superior olive (LSO), the site of origin of the LOC neurons, the main effect of disrupting LOC innervation of the cochlea via MPTP was a depression of the amplitude of the compound action potential (CAP). CAP amplitude depression was similar to that produced by LSO lesions. Latency of the N1 component of the CAP, and distortion product otoacoustic emission amplitude and adaptation were unchanged by the MPTP treatment. This technique for selectively lesioning descending LOC efferents provides a new opportunity for examining LOC modulation of afferent activity and behavioral measures of perception.

Keywords: MPTP, olivocochlear efferent, compound action potential

INTRODUCTION

The lateral olivocochlear (LOC) pathway originates in, or near, the lateral superior olive (LSO) and terminates in the ipsilateral cochlea at chemically complex synapses on dendrites of the auditory nerve (AN), and, sometimes, inner hair cells (IHCs) (for reviews, see Warr 1992; Warr et al. 1986; Eybalin 1993; Puel 1995; Le Prell et al. 2001). Given the difficulties in selectively disrupting or stimulating the LOC pathway (for review, see Le Prell et al. 2003a), little was known about LOC function until recently. One early hypothesis was that LOC efferents adjust spontaneous and sound-driven AN fiber (ANF) activity (Liberman 1990; Walsh et al. 1998; Zheng et al. 1999). Confidence in putative LOC effects was limited by simultaneous disruption of medial olivocochlear (MOC) neurons during the knife cuts to disrupt LOC neurons however. Recently, we directly demonstrated LOC modulation of AN activity by disrupting LOC neurons using LSO lesions (Le Prell et al. 2003b). Other evidence that LOC neurons modulate AN activity comes from Groff and Liberman (2003), who report electrical current at some locations within the LSO (or the ventrolateral inferior colliculus) modulates AN response amplitude. Finally, the data of

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McMahon et al. (2004), who cooled the cochlear nucleus, suggest LOC modulation of ANFs. The LOC modulation of AN activity may also be involved in the protective phenomena termed "conditioning" (see Niu and Canlon 2002; Niu et al. 2004).

Immunocytochemical evidence indicates LOC neurons contain acetylcholine (ACh), γ-aminobutyric acid (GABA), dopamine (DA), dynorphin (dyn), enkephalin (enk), and calcitonin-gene-related peptide (CGRP). Various neurotransmitters have been colocalized in cell bodies in the LSO (Altschuler et al. 1983, 1984, 1986; Abou-Madi et al. 1987; Altschuler et al. 1988; Safieddine et al. 1997) and LOC terminals (Altschuler et al. 1985; Safieddine and Eybalin 1992). While some suggest chemically distinct LOC subpopulations (Satake and Liberman 1996), others believe most LOC efferents contain all six putative LOC neurotransmitters (Safieddine et al. 1997). Consistent with broad transmitter colocalization, dopaminergic LOC neurons originate from the medial limb of the LSO (Safieddine et al. 1997; Mulders and Robertson 2004) as do neurons that show glutamate decarboxylase, CGRP, and choline acetyltransferase immunoreactivity (Safieddine et al. 1997). The medial limb of the LSO projects to the hook region and basal and second turns of the cochlea (following Robertson et al. 1987).

A neurotoxin that damages neurons containing any single LOC transmitter might produce a virtually complete LOC degeneration if LOC transmitters are colocalized. Infusing the cholinotoxin ethylcholine mustard aziridinium ion (AF64A) into the chinchilla middle ear widely disrupted LOC neurons (Smith et al. 1989; Morley et al. 1991; Smith and Mount 1993). A cholinotoxin does not selectively disrupt LOC efferents, however, as ACh is the primary MOC neurotransmitter. To disrupt LOC innervation without damaging MOC neurons, the neurotoxin should target a transmitter not contained by the MOC neurons; one such substance is DA.

The two most commonly used DA neurotoxins are 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). 6-OHDA damages catecholamine-producing neurons in the guinea pig cochlea (Eybalin et al. 1993; d'Aldin et al. 1995a; Niu and Canlon 2002). MPTP is similarly toxic to DA neurons (see Mikkelsen et al. 1999; Da Cunha et al. 2001; Petroske et al. 2001; Sedelis et al. 2001). We disrupted LOC innervation by applying MPTP to the cochlear perilymph. Synaptophysin immunolabeling revealed a reduction in LOC innervation with no disruption of MOC innervation. The functional effect of this selective loss of LOC efferents was depressed amplitude of the sound-evoked whole-nerve compound action potential (CAP). Functional measures that reflect the status of outer hair cells (OHCs) and the strength of the MOC reflex were not disrupted by MPTP.

METHODS

Subjects

Male and female guinea pigs (Elm Hill Breeding Labs, Chelmsford, MA) were used in these experiments. Functional measures included CAP and distortion product otoacoustic emissions (DPOAE; amplitude and adaptation). Tissues were harvested from a subset of these animals, as well as a small number of additional animals that did not undergo functional testing. All tissues were harvested 45 min post-MPTP. Animals were maintained with free access to food (Guinea Pig Chow, PMI Nutrition International Inc., Brentwood, MO) and water. The animal care program was AALAC-accredited. Husbandry met or exceeded all applicable standards, including the *Guide for the Use and Care of Laboratory Animals*, prepared by the National Research Council (1996). The University Committee on Use and Care of Animals at the University of Michigan approved all animal care and testing protocols.

Apparatus and procedures

CAP. CAP was recorded in anesthetized (108 mg/kg ketamine, 14 mg/kg xylazine) animals ($N = 6$) as described in Le Prell et al. (2003b). During the experiments, the tympanic membranes were examined, a tracheal tube inserted, and a platinum–iridium wire ball electrode (diameter = 0.2–0.25 mm) was placed through the wall of the cochlea into scala tympani from a postauricular surgical approach. A ball of silastic 0.5 mm distal to the end of the electrode prevented overinsertion of the electrode and loss of cochlear perilymph (as in Le Prell et al. 2004b). CAP was assessed immediately after securing the electrode in place, 30 min after round window application of an artificial perilymph solution (145 mM NaCl, 2.7 mM KCl, 2.0 mM $MgSO_4$, 1.2 mM $CaCl_2$, 5.0 mM HEPES; pH = 7.40, osmolality = 280–285 mosM), and 30 min after applying 50 mM MPTP (dissolved in artificial perilymph, pH adjusted to 7.4 ± 0.02) to the round window membrane. A subset of the animals was tested at additional longer post-MPTP time points in 30-min intervals (60 min: $N = 2$; 180 min: $N = 1$) to verify that the effects of MPTP treatment do not change within this temporal window. All round window applications were approximately 6 μl, and the middle ear was carefully dried prior to each CAP test.

Acoustic stimuli were generated using Tucker–Davis Technology (TDT; Alachua, FL) System II/System III hardware and SigGen 3.2 software. Signals were converted to analog (DA1), filtered (FT6-2, F_c =

40 kHz), attenuated (PA5), and presented using a 200-Ω transducer (Beyer Dynamic, Farmingdale, NY) coupled to the animals' ear canal via vinyl tubing. CAP input–output functions were determined for brief pure-tone stimuli (2–18 kHz; 2-kHz increments) presented at levels ranging from 0 to 100 dB SPL in 5-dB increments (5-ms duration, 0.5-ms rise–fall; 10/s). Evoked potentials were filtered (300–3,000 Hz) and amplified (1,000×) using in-house constructed equipment. BioSig 3.2 (TDT) was used to average 25 presentations within each frequency/level combination. CAP threshold was defined using linear interpolation to determine the sound level needed to produce a 10-μV response.

DPOAE adaptation. Animals ($N = 4$) were anesthetized using 1.5 g/kg urethane, then paralyzed (1.25 mg/kg tubocurarine, i.m., redosed at 2-h intervals) and artificially respirated while body temperature was maintained at 38 ± 1°C. To verify that a surgical depth of anesthesia was maintained, heart rate and blood pressure were continually monitored throughout the experiment using a digital pulse oximeter (SurgiVet Inc., Waukesha, WI).

A constant microphone position was maintained throughout drug delivery experiments. In a single animal, we compared pretreatment baseline adaptation to measures obtained after artificial perilymph application to verify that artificial perilymph applied to the round window membrane does not influence DPOAE adaptation. For all other subjects, baseline DPOAE adaptation was assessed after applying artificial perilymph to the round window membrane. In one animal, we applied three repeated treatments of artificial perilymph to verify that repeated delivery of fluids to the round window membrane did not influence DPOAE adaptation. All subjects had strong (>20 dB) baseline adaptation. We then applied 50 mM MPTP to the round window membrane (one application per animal) and reassessed DPOAE adaptation immediately as well as 30 min post-MPTP application. After confirming that MPTP did not disrupt DPOAE adaptation, we cut the OCB using a lateral brainstem cut in one animal (as in Rajan 1995; see also Liberman et al. 1996). In a second animal, MOC function was disrupted, as in Brown et al. (1969), by slow injection of strychnine hydrochloride (0.15 mg/kg, in 2.0 ml artificial perilymph solution) into the jugular vein. Strychnine is a potent antagonist of MOC function (Desmedt and Monaco 1961; Brown et al. 1969; Bobbin and Konishi 1974; Desmedt and Robertson 1975; Rajan 1988; Kujawa et al. 1992, 1993, 1994; Sridhar et al. 1995; Dolan et al. 1999; Ota and Dolan 2000).

Onset adaptation of the cubic distortion product was measured, as in Halsey et al. (2004), by using procedures modified from those of Kujawa and

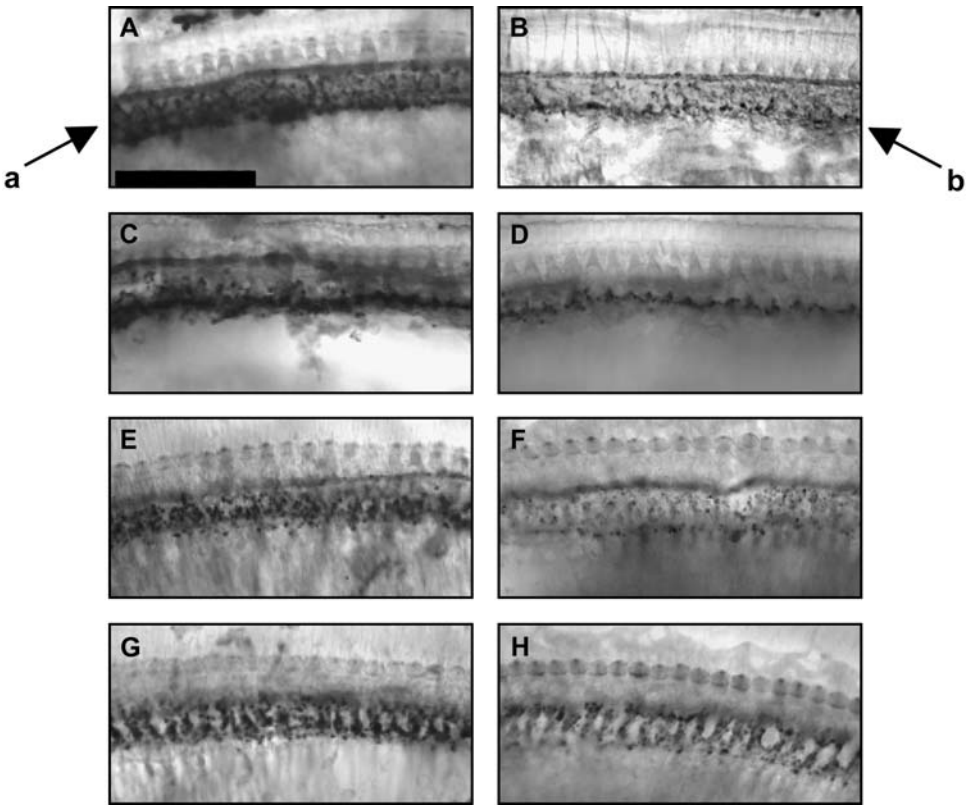

FIG. 1. Cochlear tissues had less dense immunolabeling of lateral olivocochlear (LOC) efferents after MPTP. Tissues were immunolabeled with antisynaptophysin, and are from base (**A**, **B**), second (**C**, **D**) and third (**E**, **F**) turns, and apex (**G**, **H**). Control tissues are depicted in the left panels (**A**, **C**, **E**, **G**). MPTP-treated tissues are depicted in the right panels (**B**, **D**, **F**, **H**). Arrows indicate normal LOC immunolabeling below the inner hair cells in the region of the inner spiral bundle (arrow a) and sparse LOC immunolabeling after MPTP treatment (arrow b). Scale bar = 50 μm.

Liberman (2001). Our primary tone frequencies (F1, F2) were fixed at 8 kHz (F1) and 9.6 kHz (F2); the cubic distortion product (2F1 − F2) was 6.4 kHz. F1 and F2 levels (L1, L2) were initially set to approximately 92 dB SPL; L2 was then systematically decreased over a 12-dB range in 1-dB steps. This procedure was repeated for at least six levels of F1, with F1 decreasing in 1-dB steps, until the levels producing maximum DPOAE adaptation were determined for both positive and negative deflections. To more finely resolve changes in DPOAE adaptation as a consequence of F1 and F2 levels, additional DPOAE tests were conducted with L1 and L2 level increments changing in 0.4-dB increments over at least six levels of F1, with 12 F2 levels presented for each level of F1. A MatLab program was used to control stimulus generation (TDT hardware) and presentation (Beyer sound drivers), as well as data collection.

As in Le Prell et al. (2003b), primary tones were 1 s in duration, with a 1.5-s pause between presentations. Sound pressure levels for F1, F2, and the DPOAE were determined during each level series using Fourier transform of the microphone input (Etymotic Research, ER-10B+ Low Noise Microphone). For each level combination, responses to four stimulus presentations were collected and averaged. DPOAE amplitude was sampled at 50-ms intervals during the 1-s primary tone duration. If standard deviations exceeded 2 dB at any time point, the data were excluded and the level combination was repeated. Adaptation of the DPOAE response was defined as the difference between DPOAE amplitude at the onset of the primary tones and the steady-state amplitude of the DPOAE (defined as the average DPOAE amplitude during the final four time points).

DPOAE, amplitude. Animals ($N = 4$) were anesthetized (40 mg/kg ketamine, 10 mg/kg xylazine), and the amplitude of the cubic distortion product was measured as in Le Prell et al. (2004b). The primary tones were centered at 8, 12, and 16 kHz and spaced such that F2 = 1.2 × F1. The frequency of the

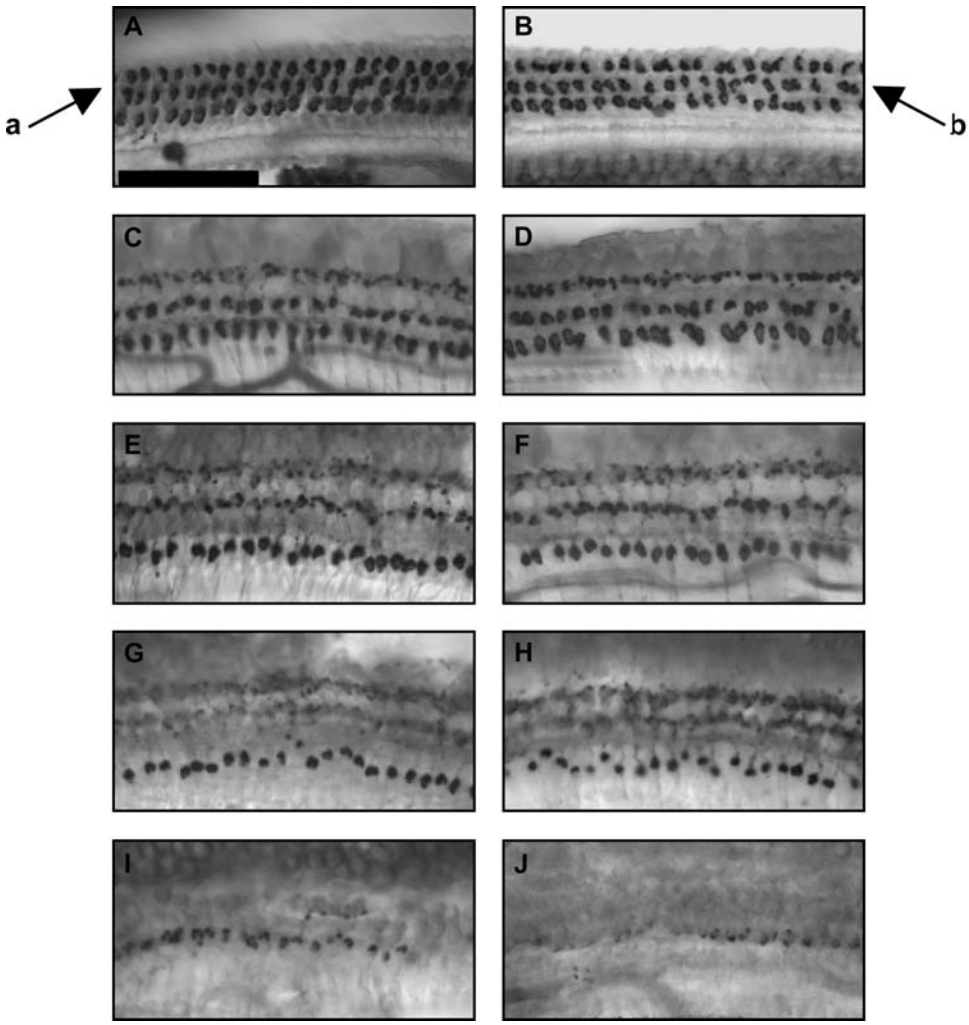

FIG. 2. Medial olivocochlear (MOC) immunolabeling was not affected by MPTP. Tissues were immunolabeled with antisynaptophysin, and are from base (**A**, **B**), second (**C**, **D**) and third (**E**, **F**) turns, lower apex (**G**, **H**) and upper apex (**I**, **J**). Control tissues are depicted in the left panels (**A**, **C**, **E**, **G**, **H**). MPTP-treated tissues are depicted in the right panels (**B**, **D**, **F**, **H**, **J**). Arrows indicate normal MOC immunolabeling below the outer hair cells (arrow a), and unchanged immunolabeling of MOC puncta after MPTP treatment (arrow b). Scale bar = 50 μm.

distortion product was equal to 2F1 − F2. Thus when the primary tones were centered at 16 kHz, F1 was 14.6 kHz, F2 was 17.4 kHz, and the DPOAE was 11.7 kHz. Initially, the level of F1 was fixed at 80 dB SPL and the level of F2 was adjusted to be 10 dB quieter. The 10-dB difference in F1 and F2 sound levels was maintained as the level of F1 was decreased in 5-dB steps, to a minimum of 25 dB SPL. DPOAE amplitude and noise floor amplitude were measured relative to F1.

Histology. At the conclusion of the electrophysiological testing, i.e., 45 min following the delivery of MPTP to the round window membrane, animals were deeply anesthetized with an overdose of sodium pentobarbital and decapitated. The temporal bones were quickly removed, dissected open at the round window and the apex, and gently perfused with 4% paraformaldehyde in phosphate buffer. Immunolabeling with antisynaptophysin mouse monoclonal antibody (1:10 dilution; IGN Pharmaceuticals, Inc.) was conducted using procedures modeled after those of Burgess et al. (1997; as in Le Prell et al. 2003b). The tissues were carefully dissected for surface preparations and mounted on glass slides. Some tissues were lost due to trauma associated with the initial gross dissection; immunolabeling was quantified in all available tissues.

For each animal, we photographed three to six regions from each available cochlear turn at two different focal planes. First, we imaged the focal plane in which LOC innervation was the densest. We then imaged MOC immunolabeling by adjusting the focal plane until MOC puncta at the bases of the OHCs were in focus. In general, within the single focal plane selected, the puncta corresponding to OHC rows 1 and 2 were the most sharply focused and the sparser MOC innervation corresponding to OHC row 3 was less sharply focused but nonetheless clearly discernable. We quantified LOC and MOC innervation using Metamorph image analysis software (version 4.6r9, Universal Imaging Corporation) as in Le Prell et al. (2003b). The LOC surface area was assessed within a 48.83 × 16.56-μm segment (total area = 800.723 μm^2) from each digitized image. The MOC immunolabeled area was assessed within a larger 76.71 × 38.83-μm segment (total area = 2,978.96 μm^2) in order to capture all three rows of MOC puncta. For both LOC and MOC puncta, we used a variable color/intensity exclusion criterion within Metamorph to measure labeling within each of the three to six sections imaged for each cochlear turn in each animal. To obtain final estimates of LOC and MOC labeling, we averaged the results from all sampled LOC or MOC regions for each animal from each cochlear turn, prior to averaging across animal data.

RESULTS

Immunocytochemistry

MPTP treatment resulted in a striking reduction in LOC terminals (38% decrease) with little or no effect on MOC terminals (10% increase). Examples of synaptophysin immunolabeling of LOC and MOC terminals in control and MPTP-treated cochleae are depicted in Figures 1 and 2. Quantitative evaluation of cochlear tissue immunolabeling is presented in Figure 3.

Statistical comparisons were conducted separately for the LOC and MOC synaptophysin quantification. Comparisons were conducted using analysis of variance (ANOVA) with between- and within-subject measures. Group means were substituted for the small number of missing data points, and the Greenhouse–Geisser correction for sphericity was applied. The within-subject measure was cochlear turn (base, turn 2, turn 3, and apex) and the between-subject variable was group (control or MPTP).

Statistical comparisons revealed LOC immunolabeling (Fig. 3A) was significantly decreased in MPTP-treated ears ($p = 0.005$); there were no reliable changes in the amount of immunolabeling as a function of

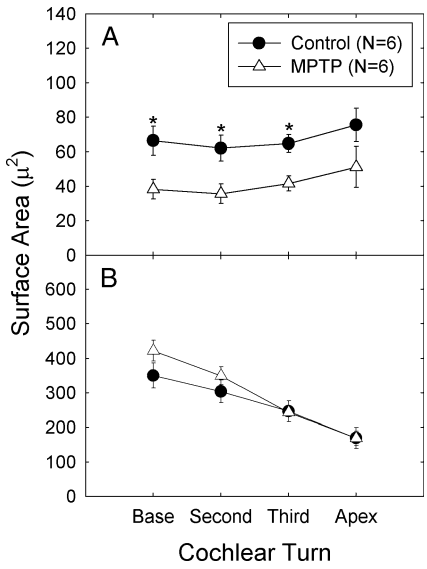

FIG. 3. **A**. Surface area of synaptophysin immunolabeling of the lateral olivocochlear (LOC) neurons was reduced in MPTP-treated cochlear tissues. **B**. Surface area of synaptophysin immunolabeling of medial olivocochlear (MOC) neurons was not reliably changed. Asterisks indicate statistically reliable differences between control and MPTP-treated ears. The amount of tissue from apical turns was not sufficient for the conduct of statistical comparisons, and all hook region tissues were excluded from analysis as the majority of hook region tissues from both the control and MPTP groups were damaged during dissection. For all other tissues, labeled area was measured in three to six regions of each cochlear turn for each animal. Labeling was first averaged within each animal; average labeling across animals is depicted.

turn ($p = 0.134$) and we failed to find a groups × turn interaction ($p = 0.932$). In contrast, MOC immunolabeling (Fig. 3B) was not reliably affected by MPTP treatment ($p = 0.170$); MOC immunolabeling significantly decreased as a function of turn ($p < 0.001$) but there was no groups × turn interaction ($p = 0.380$). Taken together, LOC immunolabeled area decreased as a function of MPTP treatment but did not reliably change from base to apex, whereas MOC immunolabeled area decreased from base to apex for both groups, but there was no difference between the control and MPTP-treated groups.

Electrophysiology

MPTP depressed CAP amplitude over the entire range of frequencies tested (see Fig. 4). The statistical reliability of drug-induced differences in CAP amplitude was evaluated using three-way repeated-measures ANOVAs. The three repeated-measures factors consisted of condition (baseline, artificial perilymph, and MPTP), stimulus frequency (2, 4, 6, 8, 10, 12, 14, 16, and 18 kHz), and stimulus intensity (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, and 100 dB SPL). Pairwise comparisons were conducted using Bonferroni corrections.

The overall analysis of CAP amplitude revealed a significant interaction of condition with frequency and intensity ($F = 3.53$; $df = 320,1280$; $p < 0.001$). When the interaction was broken down by condition, it was observed that baseline and artificial perilymph conditions were not significantly different from each other and did not interact with frequency or intensity ($p > 0.60$). Hence baseline and artificial

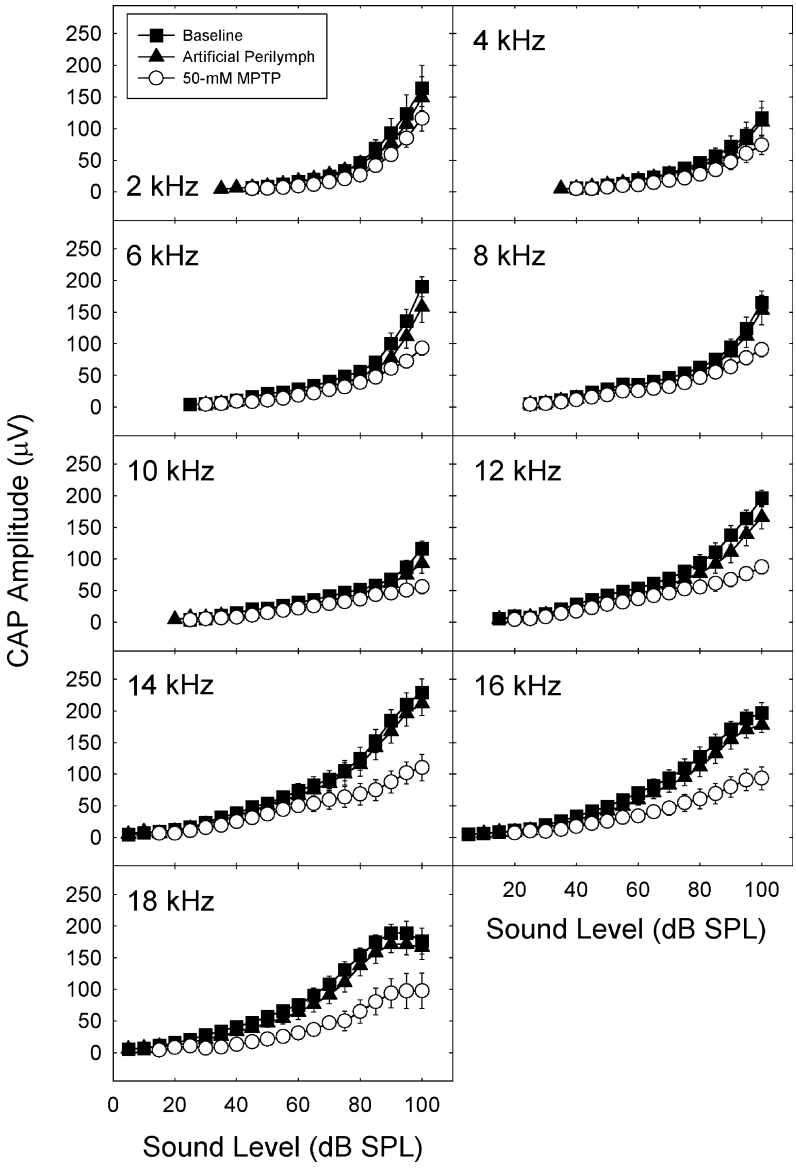

FIG. 4. Amplitude of the compound action potential (CAP) amplitude, defined as the amplitude of the N1–P1 component, was depressed after MPTP treatment. CAP amplitude was determined from 0- to 100-dB SPL at frequencies extending from 2 to 18 kHz. Mean CAP amplitude (±SE) is depicted immediately after cementing the recording electrode in place, 30 min after applying artificial perilymph to the round window membrane, and 30 min after applying 50 mM MPTP to the round window membrane.

perilymph conditions were combined and compared with the MPTP condition at each level of stimulus frequency. The contrasts of MPTP and combined control conditions interacted with intensity at all frequencies ($p < 0.01$ after applying Bonferroni correction). Thus CAP amplitude was depressed at higher intensities for the entire frequency range tested. The magnitude of the effect was smaller at the lower frequencies.

In contrast to depressions in CAP amplitude, which were observed across frequencies, MPTP induced significant elevations in CAP threshold only at higher frequencies (see Fig. 5). The frequency × condition interaction was significant ($F = 4.22$; $df = 16,80$; $p < 0.001$). After applying the Bonferroni correction, statistically reliable threshold elevations were limited to 16 and 18 kHz ($p < 0.05$).

Because threshold differences were observed at the highest test frequencies, we normalized the data to threshold by expressing CAP amplitude in decibel sensation level (SL), where 0 dB SL is the lowest signal that elicits a 10-μv neural response. The same basic effect was seen when using sensation level measures as with sound pressure level measures (not depicted). After normalizing to threshold, CAP amplitude was still depressed by MPTP at higher intensities ($F = 1.67$; $df = 40,200$; $p = 0.012$); however, this effect no longer significantly varied as a function of frequency ($F < 1$).

MPTP did not affect N1 latency (not depicted). Analysis of the latency data only revealed a significant frequency × intensity interaction ($F = 26.38$; $df = 160,800$; $p < 0.001$) that did not interact with condition.

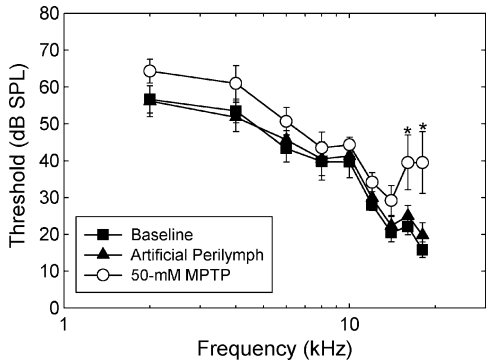

FIG. 5. Threshold (±SE) of the compound action potential (CAP) was elevated by MPTP treatment only at the highest frequencies tested. Asterisks indicate statistically reliable differences between control and MPTP-treated ears. CAP threshold was determined immediately after cementing the recording electrode in place, 30 min after applying artificial perilymph to the round window membrane, and 30 min after applying 50 mM MPTP to the round window membrane.

DPOAE adaptation

Baseline testing with the bulla opened and artificial perilymph applied to the round window membrane indicated strong (≥20 dB) DPOAE adaptation (as described by Kujawa and Liberman 2001). DPOAE adaptation, a measure of the strength of the MOC reflex (see Liberman et al. 1996; Maison and Liberman 2000; Kujawa and Liberman 2001), was essentially the same after artificial perilymph, or MPTP (see Fig. 6). Across three applications of artificial perilymph in a single animal, we observed a 2.3-dB variation in DPOAE adaptation (see Fig. 6A, B, and C). Across the four animals tested, changes in DPOAE adaptation after MPTP ranged from an increase of 4.2 dB to a decrease of 7.3 dB (mean ± SD = −2.1 ± 4.8; Fig. 6D illustrates a 3.1-dB decrease in adaptation 30 min after MPTP). In contrast to the lack of reliable changes induced by MPTP, i.v. strychnine caused a profound depression of DPOAE adaptation in the one animal tested (a 20.4-dB decrease in response amplitude; see Fig. 6E), as did OCB transection in a second animal (19.1 dB decrease in response amplitude; not illustrated).

DPOAE amplitude

DPOAE amplitude, a measure of the functional integrity of the OHC population, was not affected by MPTP (see Fig. 7). The lack of effect of MPTP on DPOAE amplitude indicates that OHC function was not disrupted by MPTP applied to the round window membrane. In contrast, euthanasia with an overdose of sodium pentobarbital profoundly depressed DPOAE steady state amplitude. The DPOAE amplitudes assessed in postmortem ears are consistent with postmortem data from rabbits (Whitehead et al. 1992) and guinea pigs (Frolenkov et al. 1998). Similar data are available from mutant (hyt/hyt) mice with OHC abnormalities (Li et al. 1999), rats treated with kanamycin (Mills et al. 1999), and guinea pigs treated with neomycin (Le Prell et al. 2004b).

DISCUSSION

These results demonstrate that MPTP, a dopaminergic neurotoxin, selectively damages LOC neurons. This is a significant technical achievement as other LOC manipulations are accompanied by simultaneous disruption of MOC neurons (i.e., Liberman 1990; Zheng et al. 1999) or auditory pathways ascending from the LSO (i.e., Le Prell et al. 2003a, b). The unmyelinated LOC neurons traveling in the OCB do not respond well to electrical stimulation

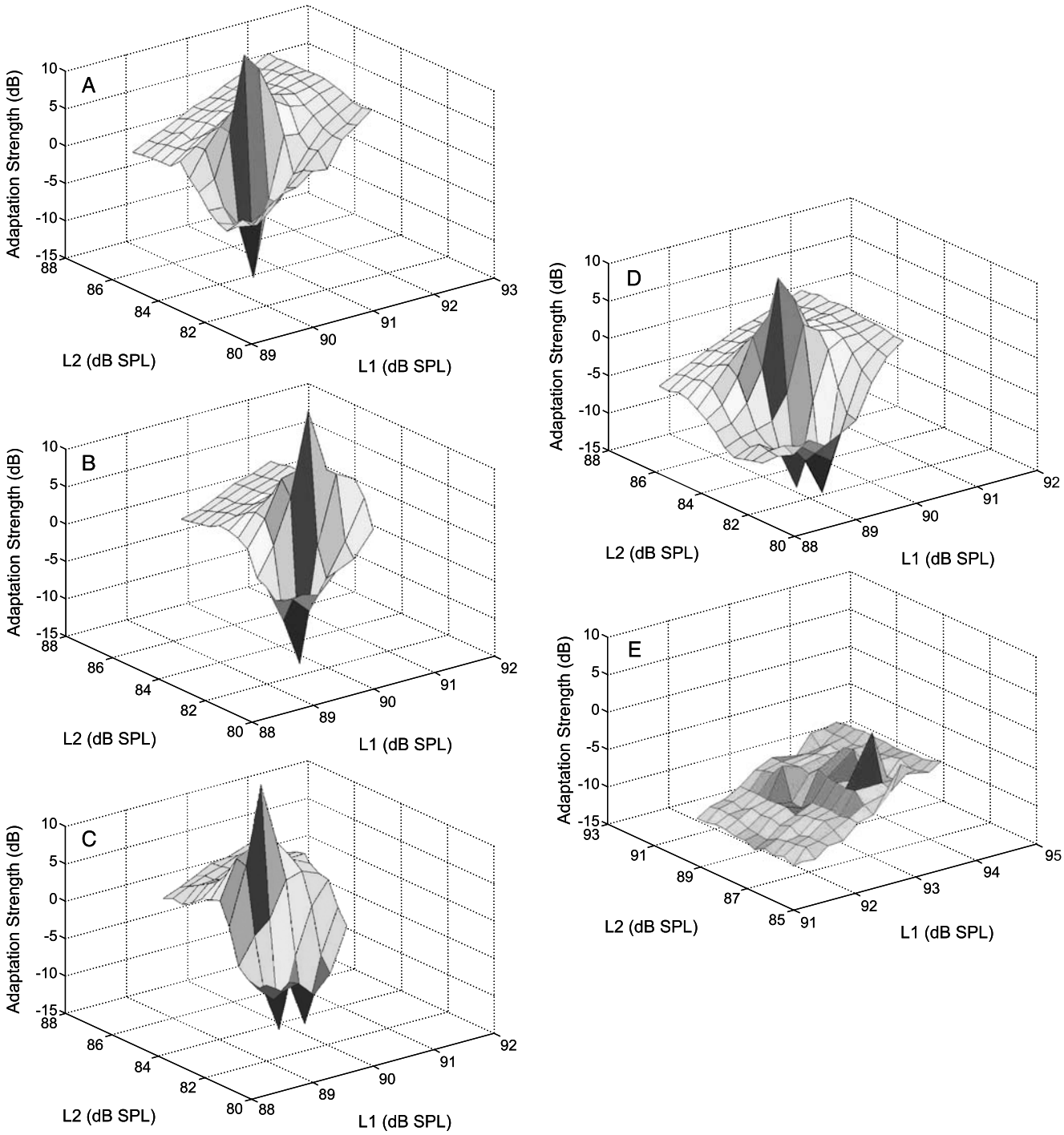

FIG. 6. Onset adaptation of distortion product otoacoustic emissions (DPOAEs) was not reliably changed by MPTP treatment. DPOAEs show a rapid level-dependant adaptation shortly after signal onset when the medial olivocochlear (MOC) pathway is intact. DPOAE adaptation is depicted 30 min after sequential applications of artificial perilymph (panels **A**, **B**, **C**), 30 min after applying 50 mM MPTP to the round window membrane (**D**), and after i.v. strychnine (0.15 mg/kg, see panel **E**).

(e.g., Guinan and Gifford 1988), and a recent effort to electrically stimulate LOC neurons via electrodes placed into the inferior colliculus or LSO yielded mixed results (Groff and Liberman 2003) with some stimulation sites producing fast MOC-like effects and others producing putative LOC effects that included site-dependent slow suppression or enhancement of CAP amplitude.

To further our understanding of LOC function, we evaluated AN activity before and after MPTP-

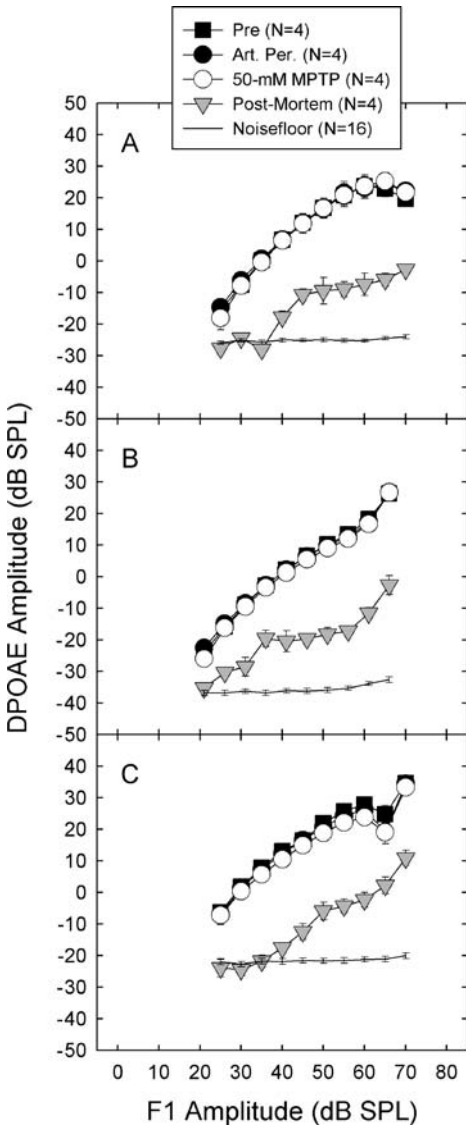

FIG. 7. Steady-state amplitude of distortion product otoacoustic emissions (DPOAE) was unchanged by MPTP treatment. Steady-state DPOAE amplitude is a measure of the integrity of the outer hair cell population. DPOAE steady-state amplitude is depicted after opening the bulla ("Pre"), 30 min after applying artificial perilymph to the round window membrane, and 30 min after applying 50 mM MPTP to the round window membrane. The levels of F1 and F2 were systematically varied such that F2 was always 10 dB quieter than F1. DPOAE frequencies were 5.8 kHz (**A**), 8.7 kHz (**B**), and 11.7 kHz (**C**).

induced LOC disruption. Consistent with the report that LSO lesions depressed CAP amplitude (Le Prell et al. 2003b), we report that LOC disruption induced by intracochlear MPTP depressed CAP amplitude. We extend our earlier data by now reporting that selective LOC disruption is not accompanied by changes in DPOAE onset adaptation or steady-state amplitude. Together, these data provide direct evidence that the LOC neurons normally modulate AN activity.

MPTP selectively disrupts LOC efferents: anatomical evidence

Anatomical evidence that MPTP selectively damages LOC neurons included a significant reduction in synaptophysin immunolabeling of LOC neurons, with no reduction in MOC labeling. Decreased LOC immunolabeling at the 45-min post-MPTP time point is not surprising as decreases in metabolic activity (Palacios and Wiederhold 1984) and mitochondrial function (Mizuno et al. 1988) as well as behavioral changes (Chiueh et al. 1984; for review, see Sedelis et al. 2001) are frequently reported within 5–30 min of MPTP injection. High-performance liquid chromatography showed MPTP to be quickly metabolized to its active form (Shinka et al. 1987), and microdialysis revealed MPTP-induced changes in DA efflux within 10 min (Wu et al. 2000). Rapid and pronounced disruption to metabolic energy presumably reduces the ability of LOC neurons to package new LOC transmitter stores, resulting in the observed decrease in immunolabeling of the protein synaptophysin (which is found in the walls of synaptic vesicles).

Lateral olivocochlear immunolabeling remaining after MPTP might indicate a small population of functionally intact LOC neurons that did not contain DA. Alternatively, MPTP treatment may have induced a functional disruption of all LOC innervation and the remaining LOC immunolabeling might be a population of neurons with intact puncta but degraded function as a consequence of the MPTP-induced mitochondrial dysfunction and oxidative stress that precede neuronal death (for reviews, see Sayre 1989; Tipton and Singer 1993; Przedborski and Jackson-Lewis 1998). Another possibility is that the remaining LOC population was preserved by one or more of the many trophic factors that reduce death of DA neurons after treatment with a DA neurotoxin. The protective effects of neurotrophic factors on AN survival (e.g., Staecker et al. 1996a, b; Miller et al. 1997; Ylikoski et al. 1998; Shinohara et al. 2002) and the presence of neurotrophic factors in the mature guinea pig cochlea (Malgrange et al. 1998; Qun et al. 1999; Stover et al. 2000, 2001; Stankovic and Corfas 2003) are well known. Glial cell-derived neurotrophic factor (GDNF) is one of the most potent trophic factors in preserving cellular function post-MPTP in the striatum and substantia nigra (for review, see Eberhardt and Schulz 2003), and GDNF is also potent in the cochlea (for review, see Altschuler et al. 1999). Resolving the chemical distribution of the intact puncta in the remaining LOC neurons, as well as overall functional status, presents an interesting direction for future investigations.

The finding that changes in LOC innervation were greatest in the lower turns is consistent with reports by other groups. For example, Mulders and Robertson (2004) report that TH-labeled neurons in the LSO were primarily limited to the medial limb of the LSO, which projects to the hook, basal (first) turn, and second turn of the cochlea. Thus they predict that an absence of dopaminergic LOC effects would be visible only on fibers well below 6 kHz. Our electrophysiological data are clearly consistent with this prediction; we report that the greatest effects were observed at and above 6 kHz. Although Mulders and Robertson (2004) report little to no TH labeling in the third turn and the apex of the cochlea, other investigations describe TH labeling in these higher turns (i.e., Jones et al. 1987; Usami et al. 1988; d'Aldin et al. 1995a; Niu and Canlon 2002). Niu and Canlon (2002) more precisely quantified TH immunolabeling in the cochlea. They reported very sparse TH immunoreactivity in the most apical turn and very dense TH immunoreactivity in the regions corresponding to 8 kHz and above (i.e., 10–11 mm from the apex). In the intermediate midcochlear regions corresponding to 1–6 kHz (i.e., 4–9 mm from the apex), TH immunoreactivity was obvious, although less dense than those regions corresponding to 8 kHz and above.

The substantial reduction in immunolabeled LOC neurons we observed, while consistent with the broad TH immunolabeling previously described in the cochlea (i.e., Jones et al. 1987; Usami et al. 1988; Niu and Canlon 2002), is seemingly contradictory to reports that the number of TH-positive neurons in the LSO is small (i.e., Mulders and Robertson 2004; Niu et al. 2004). Although the number of TH-positive neurons in the LSO is small, it is well known that at least one class of LSO neurons (termed shell neurons, in the rat) travel long distances within the cochlea, project with numerous bifurcations, and end with many terminal boutons. These neurons have been well described in the guinea pig (Brown 1987), rat (Vetter and Mugnaini 1992; Warr et al. 1997; Warr and Boche 2003), chinchilla (Azeredo et al. 1999), and hamster (Sanchez-Gonzalez et al. 2003), and may exist in the cat as well (Warr et al. 2002). Although it has not been definitively shown that TH-positive neurons in the cochlea fall within this bifurcating class of neurons, Niu et al. (2004) argue that the dense TH staining they previously observed in the cochlea (Niu and Canlon 2002) is clearly consistent with bifurcating distribution given the small number of TH-positive neurons in the LSO. Taken together, the anatomical evidence suggests the dense network of TH-positive LOC neurons in the cochlea originates from a smaller population of neurons in the LSO. Although many LOC transmitters are known to be colocalized at their site of origin (Altschuler et al. 1983, 1984, 1986; Abou-Madi et al. 1987; Altschuler et al. 1988; Safieddine et al. 1997) or their site of termination within the cochlea (Altschuler et al. 1985; Safieddine and Eybalin 1992), the precise chemical distribution within the LOC population remains to be definitively identified.

MPTP selectively disrupts LOC efferents: physiological evidence

DPOAE adaptation and amplitude were used to show MOC neurons and OHCs were functionally intact after MPTP treatment. In three of the four animals tested, we observed a small reduction in DPOAE adaptation post-MPTP, an effect that was within the variability observed across repeat applications of artificial perilymph. Although the small reductions in MOC reflex strength are within the "noise" of the repeated artificial perilymph tests, this small reduction in the MOC reflex could be predicted based on the observed reduction in CAP amplitude (i.e., Fig. 4). Depressed CAP amplitude decreases the ascending neural input that drives the descending MOC reflex loop. There were no consistent changes in OHC function, as shown in DPOAE steady-state amplitude. We previously reported no effect of MPTP on endocochlear potential (EP), indicating that the stria vascularis, which contains DA receptors (Jones et al. 1987; Usami et al. 1988; Kanoh 1995), is functionally intact (Le Prell and Bledsoe 2003).

Functional consequences of LOC disruption: MPTP vs. LSO lesion

Similar to LSO lesions (Le Prell et al. 2003b), threshold changes with MPTP were limited to a small subset of frequencies. One important difference is that LSO lesions, which consistently disrupted the lateral (low frequency) limb of the LSO, elevated thresholds at 2 kHz, whereas MPTP (applied to the round window membrane in the high frequency region, from which it diffused to lower frequency regions) elevated thresholds at 16 and 18 kHz. Threshold elevations after selective LOC disruption are an important finding. The functional status of the OHCs and the MOC neurons (which innervate the OHCs) are typically believed to set cochlear sensitivity as electrical stimulation of the crossed OCB depresses cochlear sensitivity (Galambos 1956; Wiederhold 1970; Gifford and Guinan 1987; Murugasu and Russell 1996; for reviews, see Ulfendahl 1997; Robles and Ruggero 2001). We hypothesize that LOC efferents further maintain threshold sensitivity by modulating ANF spontaneous rate. Support for this hypothesis comes from the small but statisti-

cally significant ANF threshold elevations, at a subset of test frequencies, induced by cutting both the MOC and LOC pathways (see Liberman 1990; Walsh et al. 1998; Zheng et al. 1999).

After disrupting LOC innervation, CAP amplitude was depressed. Depressed CAP amplitude was evident after normalizing the data to remove any effects of threshold changes. To directly compare the effects of LOC disruption induced by MPTP and LSO lesions, we normalized CAP amplitude for the data set shown in Figure 4 and the data reported by Le Prell et al. (2003b). The functional consequences of these manipulations were similar, particularly from 12 to 18 kHz (see Fig. 8). That the effects of MPTP were reduced at lower frequencies is not unexpected. Zheng et al. (1996) reported a similar basal-to-apical gradient of functional effects after applying kainic acid to the round window membrane in chinchillas. Similar to LSO lesions, MPTP applied to the round window did not affect N1 latency.

Pharmacology of LOC disruption: excitatory and inhibitory transmitter substances

The population of LOC neurons contains a combination of excitatory and inhibitory substances. We have proposed a model in which LOC neurons set the sensitivity of the ANFs via manipulation of a "set point," a task accomplished by balancing the release of the excitatory and inhibitory transmitter substances (see Le Prell et al. 2003a). Specifically, we proposed that delivering putative LOC transmitter substances such as ACh and/or dyn selectively lowers the cochlear set point, thereby enhancing neural activity. In contrast, release of substances such as DA and enk selectively raises the set point of the cochlea, thereby decreasing cochlear activity. Our prediction is

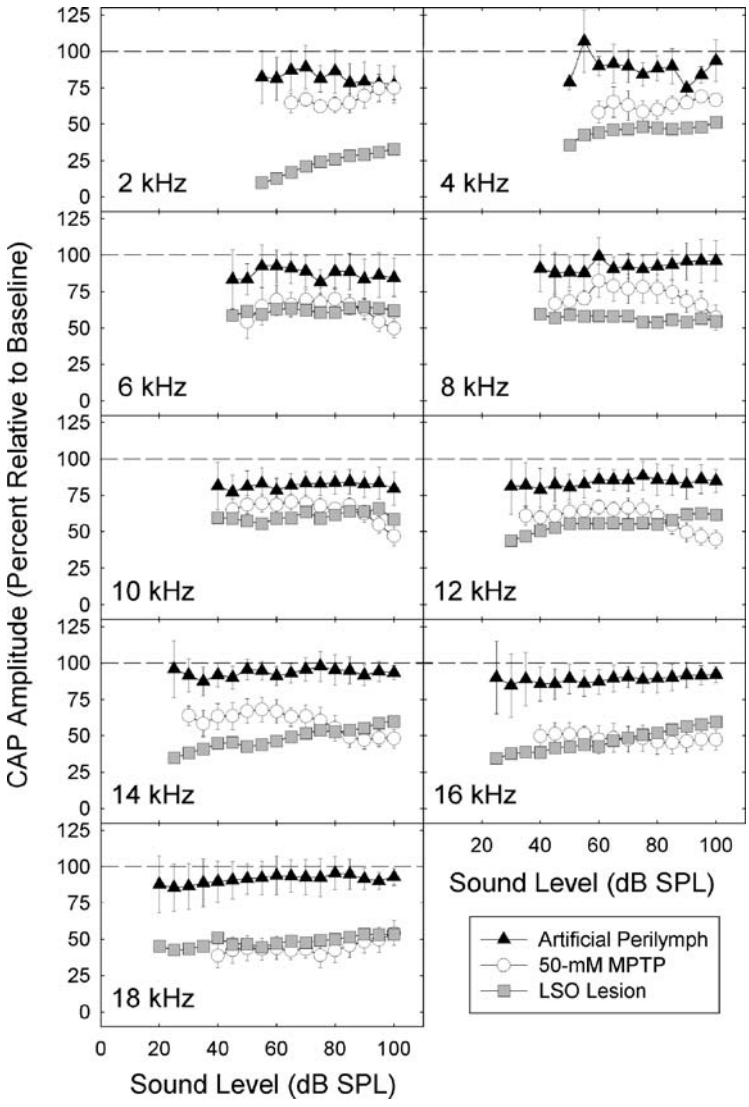

FIG. 8. Depression of the amplitude of the compound action potential (CAP) was generally equivalent in animals treated with intracochlear MPTP and animals in which the lateral superior olive (LSO) was lesioned. Amplitude of the CAP 30 min after applying artificial perilymph or 50 mM MPTP to the round window membrane was normalized to pretreatment baseline. We also normalized CAP amplitude assessed 1 week after lesioning the LSO to baseline measures from animals in which the LSO was intact (original data are presented in Le Prell et al. 2003b). Dashed lines indicate 100% of baseline; decreasing values indicate CAP was depressed post-treatment.

that this tonic input from the LOC neurons maintains the distribution of spontaneous activity and sensitivity of the ANFs in conditions of quiet and noise.

The finding that LOC disruption depresses CAP amplitude is consistent with suggestions that the net effect of LOC innervation in the cochlea is excitatory, and is consistent with the first part of the above model. Specifically, the results indicate intact LOC neurons enhance neural activity, and loss of the LOC innervation has the effect of a net depression of activity. Several LOC transmitters are candidates for enhancing AN output. ACh excites ANFs when ejected by microiontophoresis into the dendritic region beneath the IHCs (Felix and Ehrenberger 1992). We speculated that dyn may play an important excitatory role in cochlear afferent transmission (e.g., Le Prell et al. 2001, 2003a, b) based on reports that i.v. administration of the kappa agonist (−)pentazocine enhanced CAP amplitude and improved threshold sensitivity (Sahley et al. 1991; Sahley and Nodar 1994). In contrast to these earlier reports, we recently presented data showing several kappa opioid receptor agonists depress AN activity when delivered directly to the cochlea (Le Prell et al. 2004a). Thus we consider the effects of dyn in the cochlea to be unresolved, as are the effects of intracochlear CGRP. In the amphibian lateral line, CGRP increases spontaneous activity (Adams et al. 1987; Sewell and Starr 1991; Bailey and Sewell 2000a, b) and suppresses driven activity (Bailey and Sewell 2000a). There are no published reports on the functional consequences of CGRP agonists in the cochlea, although Maison et al. (2003) recently reported ABR amplitude was depressed by 20–25% in αCGRP-null mice. Depression of the ABR is consistent with an excitatory effect of CGRP on AN activity.

There is also evidence consistent with the second part of the above model. Dopamine and DA agonists inhibit AN activity (d'Aldin et al. 1995a, b; Oestreicher et al. 1997; Ruel et al. 2001; Sun and Salvi 2001). Both GABA (Felix and Ehrenberger 1992; Malgrange et al. 1997; Arnold et al. 1998) and enkephalin (Burki et al. 1993) similarly inhibit AN activity. Many hypothesize that one (or more) of these inhibitory substances are upregulated during periods of excessive (traumatic) stimulation, and LOC neurons thus protect ANFs from trauma (Pujol et al. 1993; Pujol 1994; d'Aldin et al. 1995a, b; Gil-Loyzaga 1995; Puel 1995; Gaborjan et al. 1999; Gaborjan and Vizi 1999; Halmosa et al. 2000). In fact, the DA agonist piribedil protects against hearing loss induced by exposure to loud sounds (d'Aldin et al. 1995a). In addition, limited evidence indicates that sound-evoked trauma is more pronounced in animals with LSO lesions (Le Prell et al. 2003a). In that investigation, acoustic overstimulation depressed CAP amplitude to a greater extent in lesioned animals than intact controls, an effect that was primarily limited to test frequencies lower than the sound exposure frequency.

Future directions

There is now a substantial body of evidence that the net effect of LOC innervation of the mammalian cochlea is excitatory. The pharmacological basis of this overall effect remains unclear, although several candidate transmitters have been identified. In addition, interactions among the excitatory and inhibitory substances remain to be determined. Identifying specific mechanisms through which LOC transmitters interact remains a significant challenge for future research. Our prediction is that tonic input from the LOC neurons provides a mix of excitatory and inhibitory influences that maintain the distribution of spontaneous activity and sensitivity of the ANFs. If the LOC neurons adjust the level of AN activity under varying conditions of acoustic stimulation (e.g., quiet and noise), they may provide a mechanism for the AN to maintain and accurately convey dynamic range information to the central auditory system thus maintaining an optimal set point for MOC modulation of basilar membrane mechanics.

ACKNOWLEDGMENTS

Financial support for this study was provided by NIH-NIDCD grants, including P01-DC00078 (D. Dolan, S. Bledsoe), RO1-DC004194 (D. Dolan), and P30-DC05188. We thank R. Altschuler, G. Atkin, R. Diener, L. Grant, R. Griffith, A. Kanicki, J. McLaren, S. Shubert, N. Wys, and W. Zhang for their invaluable help and assistance.

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