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Case Report

Chronic Otitis Externa Secondary to Tympanic Membrane Electrode Placement in Rhesus Macaques (*Macaca mulatta)*

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Otitis externa (OE) is a condition that involves inflammation of the external ear canal. OE is a commonly reported condition in humans and some veterinary species (for example, dogs, cats), but has not been reported in the literature in macaques. Here, we present a case series of acute and chronic OE likely precipitated by abrasion of the ear canal with a tympanic membrane electrode in 7 adult male rhesus macaques (*Macaca mulatta*). All animals displayed purulent, mucinous discharge from 1 or both ears with 3 macaques also displaying signs of an upper respiratory tract (URT) infection during the same period. A variety of diagnostic and treatment options were pursued including consultation with an otolaryngologist necessitated by the differences in response to treatment in macaques as compared with other common veterinary species. Due to the nature of the studies in which these macaques were enrolled, standard audiological testing was performed before and after OE, including tympanometry, auditory brainstem responses (ABRs), and distortion product otoacoustic emissions (DPOAEs). After completion of study procedures, relevant tissues were collected for necropsy and histopathology. Impaired hearing was found in all macaques even after apparent resolution of OE signs. Necropsy findings included abnormalities in the tympanic membrane, ossicular chain, and middle ear cavity, suggesting that the hearing impairment was at least partly conductive in nature. We concluded that OE likely resulted from mechanical disruption of the epithelial lining of the ear canal by the ABR electrode, thereby allowing the development of opportunistic infections. OE, while uncommon in macaques, can affect them and should be included as a differential diagnosis of any macaque presenting with otic discharge and/or auricular discomfort.

Abbreviations and Acronyms: OE, otitis externa; C&S, aerobic culture and sensitivity; ABR, auditory brainstem response; DPOAE, distortion product otoacoustic emission; TM, tympanic membrane; ME, middle ear; ECV, ear canal volume; URT, upper respiratory tract; nd, not determined

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Introduction

Otitis externa (OE) is a condition involving inflammation of the external ear canal.[1,19] In humans, OE can be caused by bacterial, viral, or fungal infection, dermatitis, allergic reaction, canal irritation, parasites, or secondary to middle ear infection (otitis media).[1,19] Common symptoms include otalgia, pruritis, erythema, and discharge.[1,19] Risk factors for OE in humans include canal stenosis, dermatologic conditions, water in the ear canal (swimmer's ear), and instrumentation in the canal (for example, hearing aids).[1] OE is typically managed in many species with topical or systemic agents with good success.[1]

While common in humans and in canine and feline species,[18] OE appears to be uncommon in macaques since to our knowledge, OE in macaques has not been reported in the literature. Despite being an uncommon diagnosis in macaques, guidance is warranted on OE presentation, diagnosis, and treatment options. Here, we describe a case series of 7 adult male rhesus macaques (*Macaca mulatta*, 6 to 12 y old) that presented with otitis externa.

Case Study

This case series details the presentation and treatment of OE in a cohort of adult male rhesus macaques. These subjects were enrolled in a longitudinal study (approximately 2-y duration) of electrophysiological and behavioral measures of auditory function before and after noise-induced hearing loss. After initial electrophysiologic characterization (prior to experimental noise exposure), the subjects presented with signs of OE within 7 to 42 d. OE was treated with a variety of medications and had variable durations until resolution (17 to 591 d, or unresolved). Due to the long duration of OE infections and OE-related confounds, experimental manipulations (that is, noise exposures) were not pursued, and these 7 subjects were eventually removed from the study.

Seven macaques presented with acute and/or chronic forms of OE as demonstrated by purulent, mucinous discharge from 1 or both ears ($n = 9$ infected ears). Three macaques also displayed signs of an upper respiratory tract (URT) infection (clear nasal

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discharge, coughing, and/or sneezing) during the same period. Throughout treatment, 1 macaque was found to have stenotic ear canals, which may have contributed to OE development or prolonged the duration of infection. Four of the 7 macaques had chronic cranial implants (head post ± chamber with craniotomy) at the time OE was diagnosed. Initially, potential precipitating factors for the pathophysiology of these OE infection cases included a primary contamination (for example, personnel, equipment, probes, conductive paste), a secondary contamination (for example, mechanical disruption to the epithelium, allergy), or a translocation of infection from cranial implant margins or craniotomy chambers.

All subjects underwent a standard audiologic test battery that included auditory brainstem response (ABR) testing. Tests were performed in anesthetized macaques prior to the first observation of OE symptoms. Our lab had previously measured ABRs in macaques using a recording montage with a subdermal needle electrode placed behind the ear near the mastoid. However, during these procedures, a new type of tympanic membrane electrode (TMtrode, Sanibel, Eden Prairie, MN) was inserted into the ear canal and placed on or near the ear drum to position the electrodes closer to the cochlea. These electrodes and such positioning are commonly used in animal and human subjects for ABR and electrocochleography testing. Because the TMtrode tip is similar in size to the narrowest part of the macaque ear canal, TMtrodes were placed blindly and minimal visualization was possible even with a narrow endoscope. While attempting to identify the cause of OE in these cases, and due to the lack of other precipitating events and differential causes common across subjects, we concluded that the OE likely resulted from abrasion or disruption of the epithelial lining of the ear canal secondary to TMtrode placement. Consistent with this hypothesis, ears that did not undergo ABR testing with the TMtrode did not exhibit OE. In addition, after switching back from the tympanic membrane electrode to the original subdermal needle electrode montage, no new cases of OE were noted in the colony.

Physical examination and clinical signs. OE infections were easily identified cage side by fluid drainage from the ear canal (Figure 1). Visual inspection using both a standard otoscope and an endoscope (1 mm diameter) allowed further assessment of the ear canal and tympanic membrane tissue. An endoscopic image of a healthy macaque ear canal and tympanic membrane are shown in Figure 2A, with clear orienting landmarks (umbo, manubrium of the malleus, pars flaccida) and no signs of inflammation. Inspection of ears with OE revealed obscuring fluid (Figure 2B) and erythema (Figure 2C). Tympanic membrane perforations of varying extents (partial to complete perforation) were observed in 2 ears (Figure 2D).

All macaques that were enrolled in the study underwent audiologic testing, which resulted in the OE infections in some animals. Macaques that had active OE infections and were being treated for OE were assessed at least daily for welfare and signs of pain. However, macaques with OE did not exhibit signs of pain or distress (that is, they showed no head tilt, scratching or pulling of the ears, head shaking, changes in food intake, hunched posture, changes in behavior or activity, reluctance to interact with observers, or reluctance to cooperate with experimental testing). Therefore, analgesia was not used in the treatment of these animals. Their participation in the primary study involved performing behavioral tasks and undergoing sedation for physiologic assessments. Animals with OE were withheld from active participation in the study, but were maintained as part of the study cohort in anticipation of OE resolution and possible experimental use. Once study confounds were identified through post-OE audiologic testing, the affected macaques were removed from study and euthanized shortly after audiologic characterization.

OE discharge testing and medical treatments. Subject Ju was the first to exhibit signs of an OE infection, initially observed near the end of the study timeline (the subject was to serve as a nonexposed control for comparison to noise-exposed subjects). The course of testing, treatment, and veterinary support was minimal for this case, due to the short survival time after infection and the presumption that this would be an isolated case. Subsequent OE cases occurred after subject Ju was euthanized, precluding further investigation of this infection.

Swab samples of the otic discharge were collected from the other 6 subjects for cytology testing. Cytology samples were collected in all macaques to determine OE etiology (bacterial, fungal) because this testing was conducted in-house and results could be obtained quickly. Abundant neutrophils and macrophages, as well as intra and extracellular rods and cocci, were noted on all cytology samples (Figure 3), confirming a diagnosis of suppurative, bacterial otitis. Fungi were never identified on cytology.

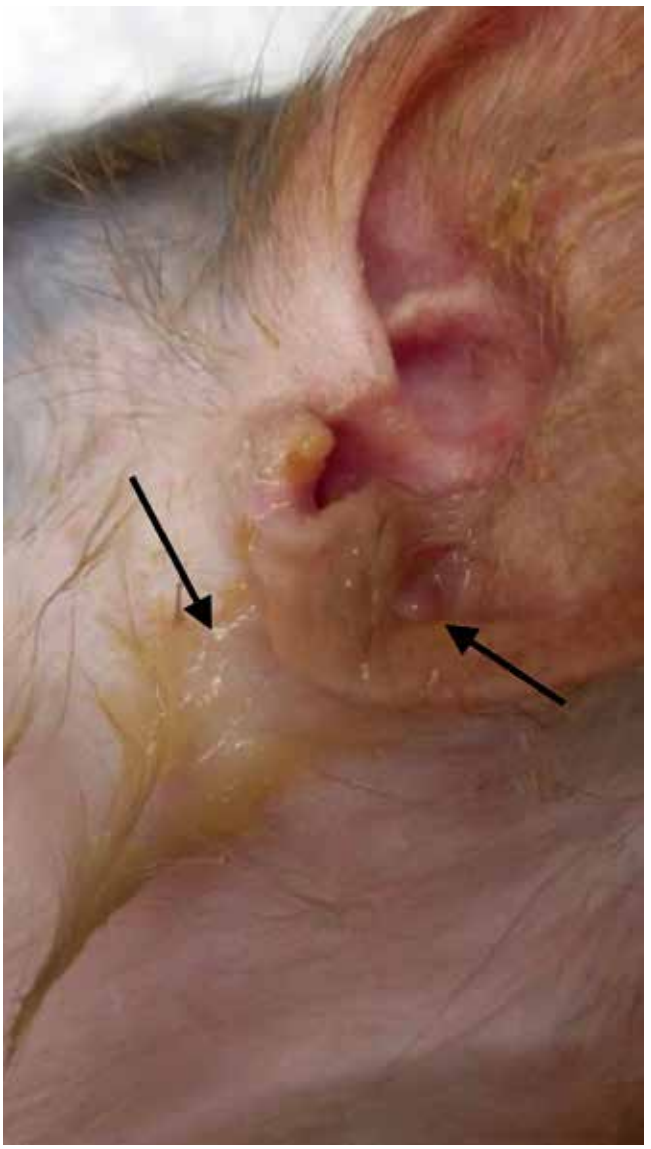

Figure 1. Profile view of macaque head showing drainage from subject Fr's left ear canal (arrows indicate drainage).

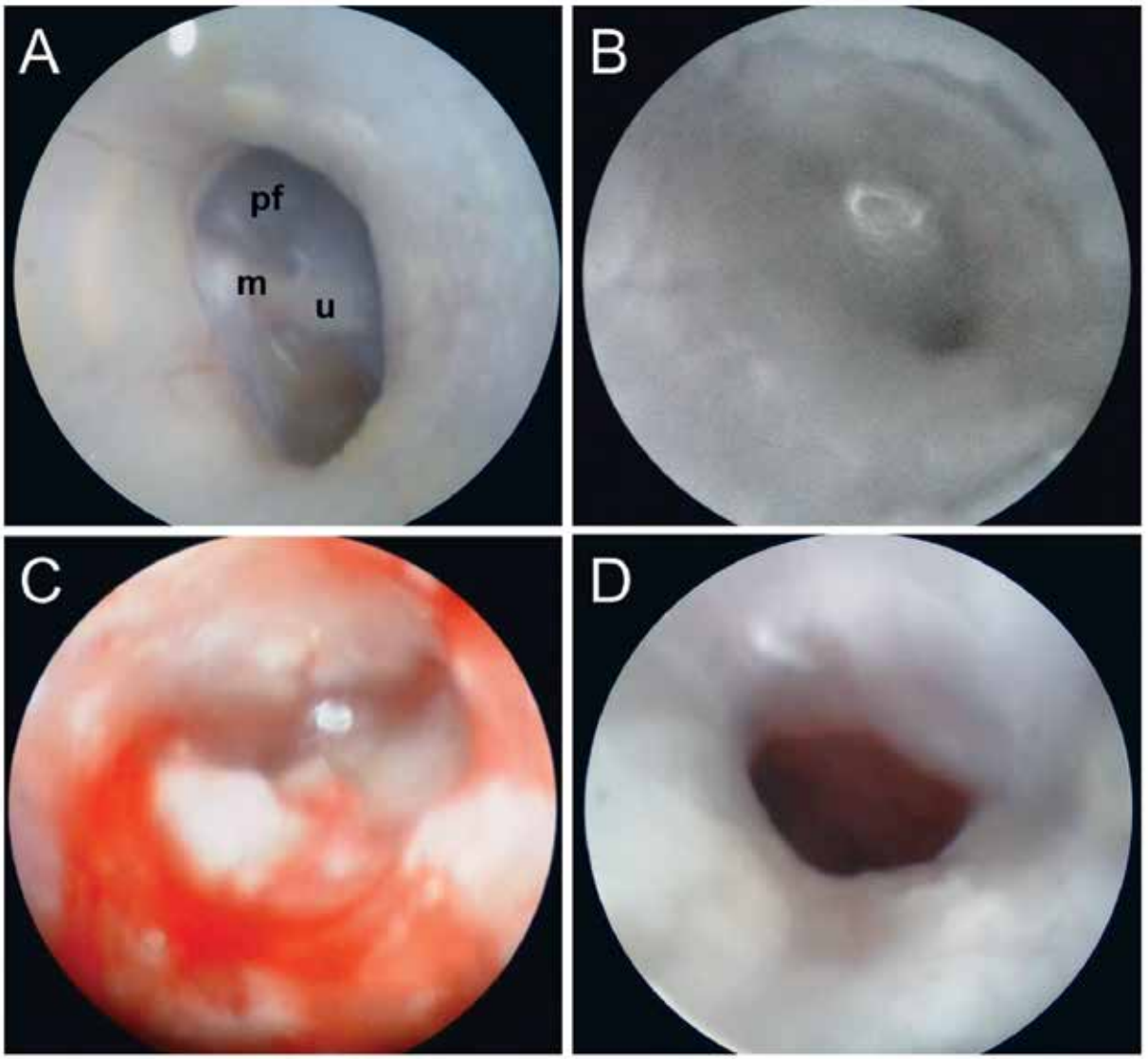

Figure 2. Endoscopic images of the ear canal and tympanic membrane in healthy and infected ears. A. Healthy ear (subject De, right ear). Orienting landmarks are visible, including the umbo (u), manubrium of the malleus (m), and the pars flaccida (pf). B. Ear with OE showing purulent, mucinous discharge (subject Ch, right ear). C. Ear with OE showing discharge accompanied by erythema of the ear canal (subject Fr, left ear). D. Ear with a complete tympanic membrane perforation secondary to chronic OE (subject De, left ear).

Additional swab samples of the otic discharge from the first 3 subjects (subjects Ch, De, Fr) were collected for aerobic culture and sensitivity (C&S) testing (Table 1). C&S testing was conducted by a reference lab (The University of Georgia Veterinary Diagnostic Laboratories, Athens, GA) and included minimum inhibitory concentrations (MICs) for antibiotics. Three subjects (Ch, De, Fr) developed URT infections concomitant with OE, which consisted of clear nasal discharge, coughing, and/or sneezing. Nasal swab samples were collected from these subjects for C&S testing. In addition, a cranial chamber swab sample was collected from subject Ch for C&S testing due to the presence of malodorous fluid in the chamber. All samples collected for C&S testing, regardless of location, showed the presence of *Staphylococcus aureus* bacteria, suggesting that this bacterium may have contributed to the occurrence of OE infections in our subjects. This likely represented an opportunistic infection as this is a common commensal organism in nonhuman primates.[20] The nose and craniotomy chamber samples from subject Ch also showed the presence of *Serratia marcescens*, which has been reported as an otitis agent in humans,[12] but is not considered a top OE pathogen.[17,24] In addition, antibiotic sensitivity testing was conducted for the suspected pathogenic cultured organisms. All bacteria were sensitive to the full panel of tested antibiotics, except subject Ch ear swab, subject Ch nasal swab, and subject Fr nasal swab, which showed oxacillin-resistant *S. aureus*.

Excluding subject Ju, the first 3 subjects to develop OE infections (Ch, De, Fr) were treated with a diverse regimen of systemic and topical drugs. Because C&S results can take up to 2 wk to receive, empirical first-choice treatments were chosen based on professional judgment and available literature. For example, Naxcel (ceftiofur; Zoetis, Parsippany-Troy Hills, NJ) was initially chosen for its broad-spectrum coverage but was discontinued for subject Ch and replaced with trimethoprim-sulfamethoxazole (TMP-SMX) because the C&S results for Ch showed oxacillin-resistant *S. aureus*. However, TMP-SMX did not resolve OE in subject Ch. Because similar organisms were found in the samples and systemic antibiotics seemed ineffective at treating OE in the first 3 cases, C&S testing was not pursued in the later cases. Topical agents, including Mometamax Otic Suspension (Merck, Kenilworth, NJ), Otic Powder (Wedge-

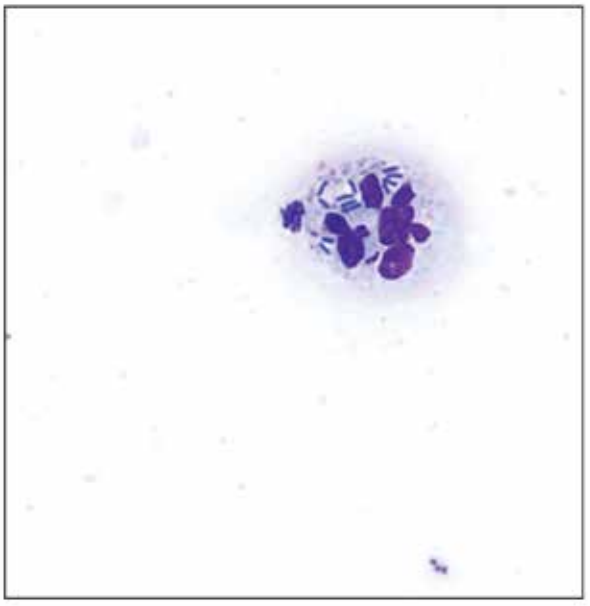

Figure 3. Representative cytologic finding from subject Fr ear canal swab showing intracellular and extracellular bacteria. All cytology samples confirmed a diagnosis of suppurative, bacterial otitis in all cases.

wood Pharmacy, Swedesboro, NJ), Neo-Predef Powder (Zoetis, Parsippany-Troy Hills, NJ), Ofloxacin Otic Solution (Rx Generics, Loveland, CO), and Claro Otic Solution (Elanco, Greenfield, IN), were tried sequentially in an attempt to identify the best treatment. Topical cleansers, including MalAcetic Otic Cleanser (Dechra, Overland Park, KS) and Debrox Solution (Lynchburg, VA), were used concurrently to flush the ear canal prior to topical treatment in hopes of improving outcomes and at the recommendation of an otolaryngologist based upon efficacy observed in human OE cases. Subsequent cases (subjects Ce, Du, El) were treated with the medications and cleansers that seemed to alleviate OE symptoms in the earlier cases. Both Claro Otic Solution and MalAcetic Otic Cleanser were applied to all infected ears (other than subject Ju and the 2 ears with tympanic membrane perforations). Claro Otic Solution contains antibiotic, antifungal, and anti-inflammatory steroid agents and is commonly used to treat canine OE infections. Due to the difficulty in managing daily ear treatments in macaques, this product was selected due to an extended time course of action after 1 dose (30 d in dogs per product label). However, pharmacokinetic and pharmacodynamic data are not available for macaques or humans for Claro Otic Solution. The URT infections were not treated with systemic antibiotics, as the clinical signs resolved quickly.

OE infection timeline. To assess the time course between significant events in the case presentations, OE infection timelines were generated for each subject (Table 2). Time from the first ABR procedure to the first observation of OE ranged from 7 to 42 d, supporting the conclusion that epithelial disruption from TMtrode insertion precipitated the OE infections. Duration of OE infections ranged from 17 to 591 d, with some unresolved cases at the time of euthanasia, indicating both acute and chronic presentations of OE. The 2 subjects that exhibited tympanic membrane perforations (De and Ce) had long infection durations (292 and 591 d, respectively), suggesting that longer durations of infection may have led to greater tissue damage and possibly development of otitis media.

Most of the subjects were treated with MalAcetic Otic Cleanser and Claro Otic Solution, so the timeline of treatment and OE resolution was examined (data not shown). OE resolution was ascertained by absence of drainage upon otoscopic examination and absence of middle ear dysfunction per tympanometry testing. The time courses were highly variable across subjects, including OE resolution prior to Claro treatment (subject De's right ear) and long duration of OE despite repeated MalAcetic and Claro application (subject Ch and Fr). These observations suggest that MalAcetic Otic Cleanser and Claro Otic Solution may have only minimally contributed to resolving the OE infections, may be more effective if used earlier in the time course of infection, and/or may require multiple applications in macaques.

Because these macaques were part of a study on auditory function, audiologic characterization of hearing status was conducted. We report the available test results from time points prior to OE and after resolution of active OE drainage.

Materials and Methods

Animals. Seven adult male rhesus macaques (*Macaca mulatta*, 6 to 12 y old) underwent audiologic testing before and after OE infection, which occurred in 9 ears (subjects De and Fr had bilateral OE). Monkeys were obtained from various sources, including the California National Primate Research Center at the University of California Davis, the Children's Hospital of Pennsylvania, and commercial vendors such as Covance. Macaques were maintained on a 12:12-h light:dark cycle with ambient temperatures of 75 °F (23.9 °C). Veterinary assessments and experimental procedures occurred between 8:00 and 17:00 during the light phase.

Three macaques were socially housed. All others were individually housed due to incompatibility for social housing, although they had visual, auditory, and olfactory contact with conspecifics in the same housing room. A commercial primate diet (Lab Diet 5037 or 5050, PMI Nutrition International, Brentwood, MO) was provided twice daily and was supplemented with fresh produce and/or foraging items (seeds, dried fruit, nuts, etc.). Macaques also received manipulanda as well as auditory, visual, and olfactory enrichment on a rotational basis. Filtered municipal water was provided at least once a day; 4 macaques were maintained on fluid restriction for study purposes.

Table 1. Aerobic culture results

Culture Site	De	Ch	Fr
Ear	*Corynebacterium ulcerans*/*Corynebacterium* spp./*Staphylococcus aureus**	*Corynebacterium ulcerans*/*Staphylococcus aureus**/*Proteus vulgaris/penneri**	*Corynebacterium bovis*/*Corynebacterium* spp./*Staphylococcus aureus**
Nose	*Corynebacterium ulcerans*/*Staphylococcus aureus**	*Staphylococcus aureus**/*Serratia marcescens**	*Corynebacterium ulcerans*/*Staphylococcus aureus**
Craniotomy Chamber	not applicable	*Staphylococcus aureus**/*Serratia marcescens**	not applicable

*Indicates sensitivity testing was performed for this organism

Table 2. Otitis externa infection timelines

	Ju	De	Ch	Fr	Ce	Du	El
Ear Affected	Right	Both	Right	Both	Right	Left	Left
First Occurrence (mm/dd/yy)	10/16/18	12/14/18	12/11/18	1/17/19	2/1/19	2/8/19	3/1/19
Initial ABR to First Occurrence (days)	18	7	23	13	42	21	35
Otitis Resolved (mm/dd/yy)	nd (not determined)	R: 12/31/18 L: nd (still present at end of study)	8/1/19	11/1/19	9/14/20	4/10/19	6/18/19
Duration of OE Infection (days)	nd	R: 17 L: 292	234	288	591	61	109

All research procedures were approved by the IACUC at Vanderbilt University Medical Center. Macaques were housed in an AAALAC International-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act and Regulations.[9,15,25]

All animals were under the continuous care of veterinary staff and received semiannual comprehensive physical exams, including standard blood work (annual) and tuberculosis testing. All cranial implants and craniotomy chambers were regularly cleaned (at least once per week, usually 5 times per week) with topical agents according to the procedures described in each IACUC approved protocol. Craniotomy chambers had been present for several years prior to OE infection and were not in active use at the time of infection.

Anesthetic procedures. Macaques were anesthetized for all audiologic testing (otoscopy, tympanometry, otoacoustic emissions, auditory brainstem responses). Initial sedation was induced with an intramuscular injection of ketamine (10 mg/kg) and midazolam (0.05 mg/kg). Animals were intubated and anesthesia was maintained with isoflurane (1% to 2%).

Tympanometry. Using an Otowave 102-4 tympanometer (Amplivox, Eden Prairie, MN), a 226 Hz probe tone was presented while a pressure sweep (+200 to −400 dPa) was generated under a hermetic seal of the ear canal. Estimates of ear canal volume (ECV), middle ear (ME) pressure, and tympanic membrane (TM) compliance were obtained. This testing was deferred for ears with active drainage and obtained after cessation of drainage.

Distortion product otoacoustic emissions (DPOAEs). DPOAE testing was completed using a Scout Bio-logic OAE System (Natus, Pleasanton, CA). Recording methods substantially overlapped with the authors' previous publications.[6,27] A probe containing 2 speakers and 1 microphone was placed in the ear. Tone pairs were presented via the speakers and emissions were recorded by the microphone. Tone frequencies were f_2 = 0.5 to 10kHz, f_2/f_1 = 1.22 presented at L_1/L_2 = 65/55 dB SPL.

Auditory brainstem responses (ABRs). ABR testing and recording methods substantially overlapped with the authors' previous publications.[6,27] Sterile subdermal needle electrodes were placed on the shoulder (ground) and forehead (active electrode). Prior to the onset of OE cases, a sterile tympanic membrane electrode (TMtrode) was placed on the ear drum and used as the reference electrode. Subdermal needle electrodes were always new, previously unused, and sterile. The TMtrodes were either new and unused in sterile packaging or were disinfected with chlorhexidine and then gas sterilized with ethylene oxide prior to use. Due to suspicions that ear canal epithelial abrasion caused by TMtrode insertion precipitated the OE infections, subsequent testing was completed with a subdermal needle electrode placed near the mastoid and posterior to the pinna as the reference. All electrodes were removed at the end of each experimental session (no chronic electrodes). Closed field speakers (MF1, Tucker-Davis Technologies, Alachua, FL;) were coupled to each ear with pediatric foam ear tips (ER3; Intelligent Hearing Systems, Miami, FL). Click and tone burst stimuli were presented (rate = 27.7 per s; 1,024 presentations, 2 repeats) across a range of stimulus levels (start at max level of 90 dB SPL, decrease in 10 dB steps) to identify the lowest sound level to evoke a measurable neural response (threshold). Physiologic data were acquired using Tucker-Davis Technologies hardware (RZ6 Multi I/O Processor, Medusa4Z preamplifier) and BioSigRZ software.

Necropsy and histopathology. After completion of the study, subjects were euthanized via overdose of sodium pentobarbital and sodium phenytoin (Euthasol, Virbac, Westlake, TX; greater than 120 mg/kg IV) and transcardially perfused with saline and 4% phosphate buffered paraformaldehyde. Temporal bones were extracted to harvest the cochlear tissue. Upon dissection, investigation of the ear canal and middle ear revealed the status of the tympanic membrane, ossicular chain, and surrounding middle ear cavity. The cochleae were perfused and decalcified for further dissection, imaging, and immunohistochemical analysis as previously described.[27] Inner and outer hair cell counts, inner hair cell ribbon synapse counts, and assessment of stereocilia condition were completed for 2 subjects (Ch and De). Data were compared with a set of normative macaque cochlear histologic data to determine hair cell integrity along the cochlear length.

Statistical analyses. Statistical analyses were conducted using linear mixed effects models ("fitlme") in MATLAB (Mathworks, 2018a). The dependent variable in the models assessing the effects of otitis externa was either DPOAE amplitude or ABR threshold. Tone frequency, individual ear, and presence of OE were entered as fixed effects into the model, while intercepts for individual macaques were entered as random effects. In all cases, *P* values were obtained by likelihood ratio testing of the model with the effect in question against the model without the effect in question. A significant *P* value was defined as $P < 0.05$. *T*-statistics are reported for each model, similar to the *F*-statistic that is often reported for such models.

Results

Tympanometry. Tympanometry was used to assess the integrity of the tympanic membrane and middle ear. Prior to OE infection, all ears exhibited normal ear canal volume, middle ear pressure, and tympanic membrane compliance (data not shown). Post-OE tympanometry was completed for 7 of the 9 ears affected by OE. Testing was deferred in cases where fluid was present in the ear canal. After resolution of active OE drainage, tympanometry was variable across subjects (Table 3). Some post-OE ears exhibited normal tympanograms (n = 2), while most displayed abnormal tympanograms (n = 5). The abnormal tympanometric findings were suggestive of persistent middle ear dysfunction (no or reduced compliance; n = 4) or tympanic membrane hypercompliance (n = 1). Post-OE tympanometry

Table 3. Tympanometry results and gross necropsy findings for the tympanic membrane and middle ear cavity

	Ju	De	Ch	Fr	Ce	Du	El
Ear Affected	Right	Both	Right	Both	Right	Left	Left
Tympanometry	not determined (nd)	R: normal L: nd, persistent drainage	R: normal ear canal volume (ECV), no measurable pressure or compliance L: normal	R: normal L: reduced compliance	R: normal ECV, no measurable pressure or compliance L: normal	R: normal L: normal ECV and pressure, hypercompliance	R: normal L: normal ECV and pressure, reduced compliance
Tympanic Membrane (TM)	nd	R: intact L: complete perforation	R: intact L: intact	R: intact L: intact	R: small perforation L: intact	R: intact L: intact	R: intact L: intact, mild scarring/opacity
Middle Ear (ME) Cavity	R: red and inflamed L: normal	R: normal L: normal, other than displaced ossicles due to TM perforation	R: normal L: normal	R: normal L: normal ME, reduced mobility of ossicles	R: almost completely filled with dense bone/ calcium deposits that embedded the stapes and malleus; no visible round window or oval window. L: normal	R: normal L: ossification in ear canal near TM, reduced TM compliance; significant ME ossification; ossicles rigidly fixed to each other and epitympanic recess	R: normal L: normal

was not completed for subject Ju. Tympanometry could not be obtained for the left ear of subject De due to persistent drainage.

Distortion product otoacoustic emissions (DPOAEs). DPOAEs were used to assess the function of cochlear outer hair cells, which provide the ability to hear soft sounds. Low-level sound emissions are produced by motility of the outer hair cells in response to pure tone pairs with specific frequency and level relationships. DPOAEs were measured in a large set of healthy ears (n = 44 to 57 ears per frequency) for tones across the macaque audible range (Figure 4A). Prior to OE, DPOAE amplitudes were within the normal range (Figure 4A). After OE, DPOAE responses were absent for all tone frequencies (Figure 4B). This was a significant reduction from the pre-OE amplitudes (t[674] = -19.5, $P = 5 \times 10^{-68}$). Uninfected ears in the same animals had normal DPOAE responses (Figure 4B; t[674] = 0.28, P = 0.78).

Auditory brainstem responses (ABRs). ABRs are a type of auditory evoked potential used to assess the integrity of the cochlea and the central auditory system, including the auditory nerve, brainstem, and midbrain. Broadband clicks and frequency-specific tone burst stimuli were used to evoke neural activity. Responses to each stimulus were collected 1,024 times for 2 repeats and averaged to identify the evoked response. Stimulus level was varied from high to low to identify the lowest level sound that evoked a neural response, defined as the ABR threshold for that stimulus. ABR thresholds were collected for a large set of healthy control ears with 2 different electrode montages: TMtrode placed on the tympanic membrane (n = 10 to 23 ears per stimulus) or needle electrode placed near the mastoid (n = 8 to 26 ears per stimulus) (Figure 5). Prior to OE, ABR thresholds were within the normal range (Figure 5A). After OE, ABR thresholds were elevated (Figure 5B) and were

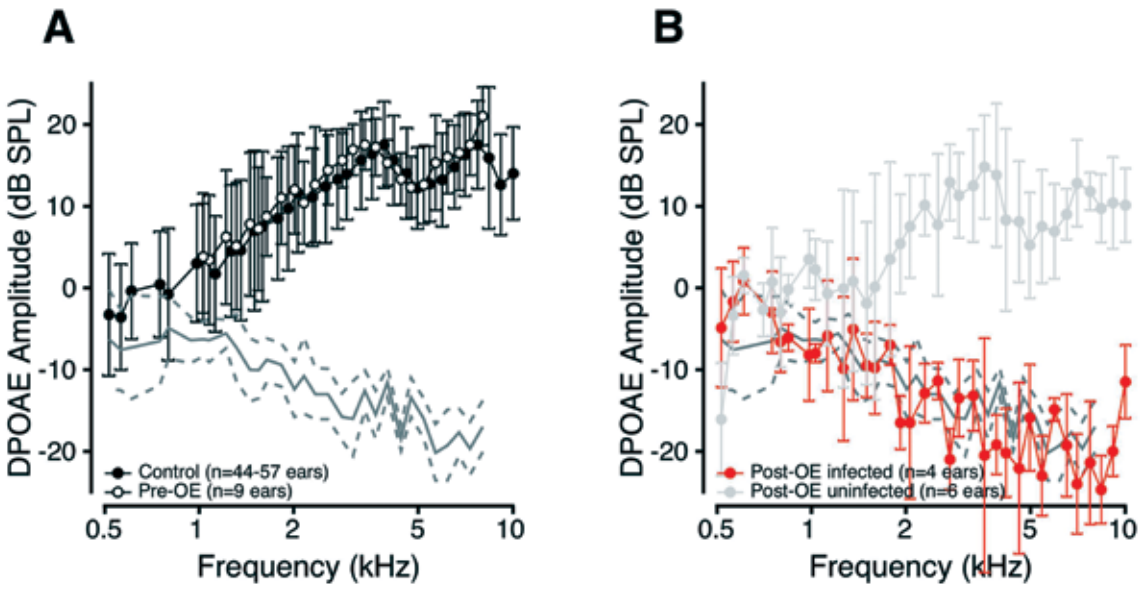

Figure 4. Distortion product otoacoustic emissions (DPOAE) testing. DPOAE amplitude as a function of f_2 frequency. Noise floor illustrated by gray dashed lines. A. Amplitudes measured before infection (black; filled = large control group, n = 44–57 at each frequency; open = pre-OE ears, n = 9). B. Ears with a history of OE had significantly reduced or absent DPOAE amplitudes (red, n = 4; within the range of the noise floor). Uninfected ears (light gray; n = 6) in the same animals had normal or only slightly reduced DPOAE amplitudes.

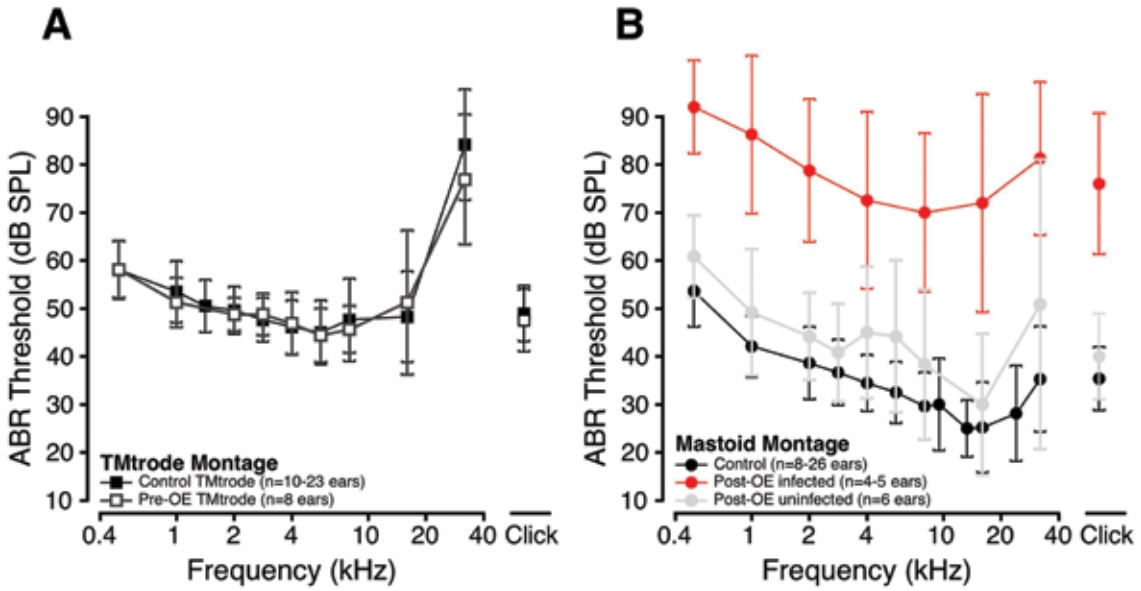

Figure 5. Auditory brainstem response (ABR) testing. ABR thresholds as a function of stimulus frequency and for a broadband click stimulus. A. Thresholds obtained using a TMtrode placed on the ear drum. Normative data (filled; n = 10 to 23 ears) and case subjects pre-OE (open; n = 8 ears) show comparable thresholds for all stimuli. B. Thresholds obtained using a subdermal needle electrode placed near the mastoid. Normative data (black; n = 8 to 26 ears) and uninfected ears (light gray; n = 6 ears) have comparable thresholds. Post-OE, thresholds are significantly elevated for all stimuli (red; n = 4 to 5 ears).

significantly different from pre-OE values (t[176] = 12.49, $P = 5 \times 10^{-26}$), suggesting poor hearing sensitivity in these ears. Uninfected ears in the same animals had normal ABR thresholds (t[176] = −1.07, P = 0.29).

Necropsy and histopathology. After euthanasia, ear canals and middle ear cavities were dissected to permit cochlear perfusion for histologic investigations. Consistent with prior otoscopic and endoscopic examinations, tympanic membranes (TMs) were intact for all subjects except Ce and De, who had partial and complete perforations of the TM, respectively. Middle ear abnormalities were observed in 4 of the 9 ears with a history of OE infection (Table 3). Abnormalities included erythema and inflammation (subject Ju; acute OE), reduced ossicular mobility (subject Fr left ear, chronic OE), and ossification of the middle ear ossicles and cavity (subjects Ce and Du; chronic OE). These observations are consistent with the tympanometric findings, as reduced compliance typically accompanies ossification.[5] Abnormalities of the tympanic membrane, ossicular chain, and middle ear cavity likely contributed to the hearing dysfunction measured with the DPOAE and ABR testing in the form of a conductive hearing loss. Cochlear histology was completed for both ears from subjects Ch and De. Inner and outer hair cell counts, inner hair cell ribbon synapse counts, and hair cell stereocilia conditions were not significantly different from normal hearing control subjects at any cochlear frequency place (data not shown). This finding also suggests that the hearing loss measured via DPOAE and ABR testing was primarily conductive (as compared with sensorineural) in nature. Contributions from cochlear damage cannot be ruled out in the remaining cases.

Discussion

Here we described the auditory function of 7 macaques before and after OE. Ears with a history of OE infection showed persistent middle ear dysfunction, as assessed by tympanometry and necropsy. DPOAE and ABR testing indicated significant hearing loss in all ears with a history of OE infection. Cochlear histopathology from 2 subjects revealed intact inner ear structures, suggesting that the hearing loss was likely conductive in nature (that is, sound conduction from the outer and middle ear to the inner ear is reduced). In summary, chronic OE can lead to permanent decrements in auditory function, even after apparent resolution of OE signs. For this reason, infections should be treated immediately and aggressively.

Treatment challenges and recommendations. Developing diagnostic and treatment plans for the macaque OE infections was challenging, in part due to the lack of literature available regarding different treatment options. Although otoscopic exams and C&S and cytology testing are routine for veterinary medicine, diagnosis was hindered by the need to sedate for ear exams and swab sample collection for cytology and C&S testing, as well as the time to receive test results. Conventional methods used to treat OE in humans and other animals, such as daily ear cleanings and topical treatments, were also limited by the need to perform cleanings and apply medications under sedation. The delay between OE onset, sample acquisition, and test results complicated and delayed the informed selection of topical or systemic antimicrobial agents. Compounding the challenges of antibiotic selection was the paucity of published data regarding antibiotic resistance of bacterial agents sampled from macaques. In addition, most medications used in macaques are off-label and lack sufficient pharmacokinetic or pharmacodynamic data to guide their use.

Although otoscopic exams were performed regularly while the animal was sedated for treatment, extensive fluid drainage made it difficult to assess the condition of the tympanic membrane (TM) and precluded tympanometry testing. Furthermore, many of the common veterinary medical products used to treat ear infections contain at least 1 active ingredient that can be ototoxic (for example, gentamicin, neomycin) and could interfere with the primary research objectives. Claro has only been tested for use in dogs, so ototoxicity in macaques has not been assessed. However, the hearing loss and experimental delay caused by the OE infections still presented a significant experimental confound

to our studies. Because the macaque OE pathophysiology and access to daily treatment differed from typical veterinary species (for example, dogs or cats), an otolaryngologist was consulted regarding endoscopic ear canal examinations and guidance for diagnostic and treatment regimens.

OE treatment in macaques should begin at the first sign of infection and occur as frequently as possible until the infection is resolved. Sterile 0.9% saline is preferred to the use of topical otic cleansers (for example, MalAcetic Otic Cleanser), due to its ability to be used safely in the presence of a perforated TM. If the TM can be confidently diagnosed as healthy and uncompromised, otic cleanser may be used. Claro Otic Solution was another preferred treatment option for our macaque OE cases due to its long duration of action (approximately 30 d in dogs; unknown in macaques). This was useful for minimizing repeated sedations for administration of treatment. However, the poor response to Claro treatment may indicate a need for more frequent administration in macaques as compared with dogs. In addition, Claro should not be used if TM perforation is suspected. In these cases, 0.3% Ofloxacin Otic Solution can be used as an alternative, as it is safe for the middle ear.

Topical steroids are used in veterinary medicine to treat animals with OE secondary to allergies by decreasing inflammation in the ear.[21] Two of the topical treatments we used contain glucocorticoids (Claro and Mometamax; both contain mometasone furoate) and may have been beneficial in treating these cases of OE secondary to experimental manipulations. However, we did not pursue long-term treatment with these agents for several reasons. First, steroids inhibit the innate immune response and are not safe for long-term use due to increasing tolerance and eventual resistance. Second, topical steroids can lead to skin thinning, changes in natural cortisol levels of the body, and, if ingested, GI distress (ingestion is highly likely after topical use in macaques).[3,7,16,21] Third, many topical steroids contain alcohol, which is contraindicated in ears with TM perforations.

In other species, OE is commonly associated with ear pain. In these cases, analgesia is recommended to treat this chronic discomfort. However, we did not observe signs of pain or distress in our macaques with OE during daily monitoring. For this reason, analgesia was not provided for our macaques, but would be recommended if signs of pain were present in other presentations of OE.

Possible etiologies of OE and associated hearing deficits. Acute OE can be directly caused by primary factors, such as foreign bodies and trauma to the epithelium of the ear canal.[14] All macaques that underwent ABR testing with a TMtrode placed in the ear canal near the TM contracted OE, suggesting that abrasion or disruption of the epithelium was the primary cause. The alternative ABR testing approach using subdermal needle electrodes was not associated with OE and represents a refinement of methodology if a TMtrode is not required for specific experimental questions. The discontinuation of TMtrode use in combination with periodic otoscopy and tympanometry guarded against future infections. OE can become chronic in response to perpetuating factors, such as bacterial infection, progressive pathologic changes in response to chronic inflammation (for example, skin thickening, canal stenosis), or otitis media.[14] *Staphylococcus aureus* bacteria, a common commensal organism in nonhuman primates,[20] was present in all ear swabs, and may have caused a perpetuating, opportunistic infection during OE. Stenosis was noted in 1 macaque and may have perpetuated OE in that case. Finally, otitis media may have been present in some cases, but the purulent, mucinous fluid drainage prevented confirmation due to limited visibility of the tympanic membrane and the inability to perform tympanometry testing. Otitis media is known to perpetuate chronic OE, even with an intact tympanic membrane.[14]

In addition, otitis media is often concomitantly observed with URT infections in cats and other species, and URT infections were observed in 3 of the 7 macaques. The cause of the URT infections was difficult to determine, as no aerobic bacterial agents that could explain the upper respiratory signs were identified on C&S (see Table 1) and our study did not explore viral etiologies. However, because the clinical signs of the URT infections resolved quickly, were not present in every animal, were not temporally related to development of OE, and no additional OE cases developed after the TMtrode was no longer in use, we concluded that URT infections were likely not the primary cause of the OE cases.

Chronic otitis media can lead to fibrosis, calcification, vascularization, and erosion of the middle ear space and ossicles, and even the inner ear in some cases.[22,23] Necropsy results showed middle ear inflammation in subject Ju, reduced ossicular mobility in the left ear of subject Fr, and middle ear ossification in subjects Ce and Du, suggesting possible chronic otitis media in these cases.

TM perforations, altered TM and middle ear compliance, and middle ear ossification in the presence of elevated ABR thresholds and absent DPOAEs suggests a diagnosis of conductive hearing loss. This conclusion is further supported by the normal cochlear histology observed for subjects Ch and De. However, sensorineural components cannot be ruled out in the remaining cases. The severity of hearing loss approaches the maximum conductive hearing loss possible, as high-level sounds (> 60 dB SPL) can be conducted through the bones of the skull.[26] Chronic otitis media has been reported to lead to sensorineural hearing loss in some cases.[4,5,23]

Chronic conductive hearing loss is typically well-managed in humans through bone-conduction amplification devices. However, these treatment options are limited for research animals. From a research perspective, chronic conductive hearing loss is a form of auditory deprivation, which can alter physiologic responses and organization of the central auditory system and other brain areas[10,11,13,29] and cause changes in auditory perception.[2,8,28] Therefore, rapid diagnosis and treatment of OE is key to avoiding these scientific confounds. Environmental accommodations such as increased visual, olfactory, and somatosensory enrichment are also recommended to support the wellbeing of animals with hearing loss.

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