Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Time-domain and frequency-domain effects of tensor tympani contraction on middle ear sound transmission in gerbil

Liam Gallagher^a, Mohamed Diop^a, Elizabeth S. Olson^{b,*}

^a OTO/HNS, Columbia University Medical Center, New York, United States

^b OTO/HNS and BME, Columbia University, 630 W 168th street, New York, NY 10032 United States

ARTICLE INFO

Article history: Received 12 January 2021 Revised 17 March 2021 Accepted 20 March 2021 Available online 8 April 2021

Keywords: Middle ear Tensor tympani Umbo motion Pulse train stimulus Zwuis multitone Click Gerbil auditory system

ABSTRACT

The middle ear is a high-fidelity, broadband impedance transformer that transmits acoustic stimuli at the eardrum to the inner ear. It is home to the two smallest muscles in mammalian species, which modulate middle ear transmission. Of this pair, the function of the tensor tympani muscle (TTM) has remained obscure. We investigated the acoustic effects of this muscle in young adult gerbils. We measured changes in middle ear vibration produced by pulse-train-elicited TTM contraction – in the time-domain with a click stimulus and in the frequency-domain with multitone zwuis stimuli. In our click experiments, there was generally a small reduction in the primary peak of the response and a slight increase in the subsequent ringing, but there was essentially no change in the delay of the click response at the umbo (less than 1 μ s change). In our multitone experiments, there were consistent patterns of attenuation and enhancement in the velocity responses at the umbo and ossicles. TTM contraction produced a narrow band of enhancement around 6 kHz (maximally ~5 dB) that can be modeled with an increased stiffness of an overdamped spring-mass resonance. At frequencies below 2 kHz and above 35 kHz, TTM contraction attenuated middle ear vibrations by as much as fivefold.

© 2021 Elsevier B.V. All rights reserved.

1. Introduction

The middle ear efficiently conducts sound from the air of the ear canal to the fluid of the cochlea, through membrane, bone, and joint. The movements of the eardrum and ossicles are complex, vet the sound stimulus at the eardrum ends up quite faithfully reproduced in the motion of the stapes at the oval window (Fay et al., 2006). However, the middle ear is not a purely passive impedance transformer. The two muscles of the middle ear - the stapedius muscle and the tensor tympani muscle (TTM) - apply loads to the ossicles that change the way they move, thus modulating the signal that is delivered to the inner ear. In human, contraction of the TTM exerts an anteromedial force on the handle of the malleus producing an inward deflection and tensing of the tympanic membrane. The stapedius applies a posterior load on the neck of the stapes, stiffening the annular ligament around the footplate. Traditionally, these anatomically antagonistic muscles were thought to be functionally synergistic and to comprise the acoustic reflex (Gelfand, 1998). Over time, however, the acoustic reflex has come to be understood as the stapedial reflex, with the TTM playing a

* Corresponding author. E-mail address: eao2004@cumc.columbia.edu (E.S. Olson). minor role or no role at all (Gelfand, 2009). The function of the TTM has remained a subject of interest and some mystery.

There are numerous theories about the function of the TTM, many of which are as old as our knowledge of the muscle itself. In 1600, a few decades after the muscle was originally described by Eustachius, the first theory of the function of the muscle was proposed: that it serves to protect the eardrum from loud sounds (Borg et al., 1984). Not much later, the idea emerged that the muscle might selectively tune the ear to certain frequencies (Borg et al., 1984). Others offered the analogy of the ciliary muscle of the eye, suggesting that the TTM contributes to the dynamic range of audition through accommodation. Over the decades and centuries, important theories of TTM function and mechanism have emerged and evolved. These include: 1) protection against injurious sounds; 2) frequency-tuning; 3) ossicular support/fixation; 4) labyrinthine pressure control; 5) pneumatic Eustachian function; 6) sound localization; 7) dampening self-generated sounds (Borg et al., 1984). Each of these theories has sought to integrate our knowledge of the muscle's anatomy, triggers, neuronal input, and its effect on hearing in humans and important animal models.

Relative to the neural circuit of the stapedial reflex, the neuronal inputs to the TTM are complex (Mukerji et al., 2010). This complexity is consistent with observations that the TTM contracts with not only intense sounds, but also self-generated sounds,



Research Paper





non-auditory stimuli, and even voluntarily (Møller, 1984). In contrast, case reports of voluntary control of the stapedius muscle are notably absent from the literature. Although the TTM is less acoustically active than the stapedius, anticipation of a loud noise or startle can cause contraction (Djupesland, 1964). Nonauditory triggers such as forceful eye closure and orbital air jets have been shown to produce strong TTM contractions in humans (Rock, 1995)(Bance et al., 2013). As a muscle innervated by the mandibular nerve, it has been argued that the TTM should be considered a muscle of mastication and that it is responsible for attenuating sounds produced by eating and vocalization (Ramírez et al., 2007). In cat, the TTM contracts in anticipation of vocalizations and also with large bodily movements (Galambos & Rupert, 1959)(Carmel & Starr, 1964). Although the muscles in the group innervated by the mandibular nerve are traditionally referred to as the muscles of mastication, these also include muscles involved in Eustachian tube function, notably the tensor veli palatini. In fact, the TTM is intimately related to the tensor veli palatini, and some have suggested that the two muscles may act in concert to open the Eustachian tube and normalize middle ear static pressure (Honjo et al., 1983).

Observations of individuals with voluntary control of the muscle were some of the earliest investigations and indicated that the muscle might have a frequency-dependent effect. As early as the 19th century, Politzer observed the effects of the TTM on sound transmission: "during the contractions the hearing distance was diminished, the deep tones became deadened and indistinct, and the high tones ascended about one quarter of a tone" (Politzer, 1909). When audiometry became available, quantitative analysis of voluntary TTM contraction was shown to be consistent with Politzer's earlier qualitative impressions (Smith, 1943). Other more recent case reports and case series of individuals with voluntary TTM control have repeated earlier findings that the muscle can produce low-frequency hearing loss and tinnitus, often described as "cracking" or "rumbling" (Angeli et al., 2013)(Wickens et al., 2017).

Experimental investigations of the acoustic effects of the muscle have been conducted in cadaveric human temporal bones, cat, rabbit, and guinea pig. In cadaveric human temporal bones, sectioning the TTM had minimal effect on umbo displacement, suggesting that the muscle does not exert a passive effect in the absence of contraction (Gyo & Goode, 1987). More recently, static pulls on the TTM in human cadaveric temporal bones were reported to produce a six-fold decrease in sound-driven umbo displacement and 200 µs reduction in middle-ear delay at frequencies below 1kHz (Cho et al., 2019). This 200 µs delay represents a significant fraction of the maximum interaural delay which might indicate that the muscle plays a role in enhancing lateralization cues.

There is a long history of experimental investigations in animal models focused on both middle-ear muscles (MEMs) and the TTM specifically. Many early experiments measured the cochlear microphonic or middle ear impedance (Avan et al., 1992). In one report, cochlear microphonic in unanesthetized cats was measured chronically over months after cutting the middle-ear muscles on one side (Galambos & Rupert, 1959). Cochlear microphonic in the intact ear was attenuated relative to the side with the muscles cut when the sound stimulus exceeded the amplitude threshold required to evoke MEM contraction. Isolating the two muscles indicated that the stapedius was primarily responsible for these effects. Similar experiments in rabbit produced similar findings: that attenuation of cochlear microphonic was primarily due to the stapedius muscle and greatest at low frequencies (Borg, 1972b). In guinea pig, changes in middle ear impedance with spontaneous contractions of the middle ear muscles indicated that the muscles attenuate low frequencies but have complex effects at higher frequencies (Funnell, 1972).

Nuttall measured cochlear microphonic in guinea pigs while eliciting TTM contraction with a voltage pulse train stimulus, where earlier experiments had relied upon the acoustic reflex, spontaneous contractions, or the application of artificial tension to the tendons of the MEMs (Nuttall, 1974). The use of electrical stimulus to contract the TTM allowed for specific and physiological contraction of each MEM. Each muscle produced frequencydependent effects on the cochlear microphonic. The studies of Nuttall followed decades of observations in which contraction-induced changes in cochlear microphonic have been used to assess middleear sound transfer (Borg, 1972a)(Møller, 1964)(Møller, 1965).

The Mongolian gerbil, meriones unguiculatus, has served as an important model animal for study of the mammalian auditory system, for example (Wolf et al., 2010)(Lenarz, 2002)(Dong & Olson, 2013)(Carney et al., 2011). The audible frequency ranges of the human and gerbil are reasonably similar, although the gerbil's range extends to higher frequencies (Rosowski et al., 1999)(Ryan, 1976). Mongolian gerbils, like other desert mammals, have relatively large middle ear spaces which facilitates surgical investigation of the muscles and other structures therein (Mason, 2016). Many gerbil vocalizations are ultrasonic (higher than 20kHz), and hearing sensitivity at those frequencies is affected by their surroundings (Nishiyama et al., 2011). Specifically, experiments have shown enhancement in the cochlear microphonic response, but only when a gerbil was alert and interacting with a companion gerbil (Miyawaki et al., 2015). The mechanism of this enhancement has not been described.

The study here used two different types of broadband stimuli in two separate sets of experiments in two separate sets of animals. The broadband nature of the stimuli was important because it allowed for rapid data collection during the time when the muscle was electrically stimulated. Thus, any time-dependent changes in TTM contraction would occur at all the frequencies concurrently and not introduce a frequency-dependent bias. Clicks were our first broadband stimuli. Transient sounds like clicks, fricatives, and sharp rustling noises are common sounds whose perception and localization is important for communication and survival. Significant changes to transmission upon TTM contraction such as changes in transmission delay or changes in response persistence might be more apparent when visualized in the timedomain. Moreover, the click stimulus and response can be cast into the frequency domain to find the frequency-dependent transfer function. However, the click was not a perfectly flat broadband stimulus and contained less sound energy at frequencies below ~2 kHz and above ~35 kHz. The second set of experiments used zwuis multitone stimuli, calibrated to provide a uniform sound pressure level at each of the included frequencies. We used three different multi-tone zwuis probes, with low, medium and wide-band frequency representation.

The experimental approach employed in this report can be understood as an evolution of that employed by Nuttall, using a voltage pulse train to stimulate the muscle. Our experimental priorities were to: 1) produce a physiological, near-maximal contraction of the muscle; 2) probe with a broadband sound stimulus; 3) prepare an acoustically intact middle ear space; 4) use vibrometry to measure middle ear motion changes at points along the chain of transmission. A desire for realism guided these priorities. Delivering an electrical stimulus to the muscle produced a highly repeatable, contraction-mediated load on the malleus. Using click and zwuis sound stimuli enabled measurement of time-domain and frequency-domain responses with a full-strength muscle contraction. Opening the bulla is known to affect middle ear transmission (Ravicz et al., 1992), particularly at low frequencies. It is also at low frequencies where the effects of TTM contraction are expected to be large. In our set of multitone experiments we sealed the bulla after electrode placement to more fully investigate the frequency-



Fig. 1. A. Click stimulus measured just outside the ear canal. B. Frequency domain representation. Expt 4-30-19 run 3.

structure of TTM contraction. Finally, most investigations on the effect of the TTM have focused on cochlear responses, which are indirect measurements of middle ear vibration, so it was important to elucidate the more proximate effects the TTM has on the movement of the eardrum and ossicles.

2. Methods

We present results on the effect of TTM contraction on middle ear transmission from 13 young adult gerbils, aged 2-3 months and weighing between 40 and 60 grams. The experiments using a click stimulus were numbered (dated): 4-9-19, 4-17-19, 4-30-19, 5-6-19. The experiments using zwuis stimuli were numbered (dated): 7-30-20, 8-13-20, 9-10-20, 9-18-20, 10-5-20, 10-14-20, 10-16-20, 11-3-20, and 11-13-20. Several additional gerbils were used in the development of the study.

2.1. The sound stimulus

Two different broadband sound stimulus types were used, click and multitone. In all experiments, the sound stimulus was delivered open-field ~1cm from the ear canal and measured by the microphone, which was placed just outside the ear canal, within ~5 mm of the eardrum. The first set of experiments used a click. To create the acoustic click, a TDT (Tucker Davis Technologies, Alachua, Florida) RX6 signal processor, sampling at 5 µs, produced a voltage that went from 0 to a peak value in 5 µs, stayed up for two samples and then ramped back down to 0. This voltage signal drove a TDT HB7 headphone buffer, which drove a IIX earbud. This resulted in an acoustic click ~ 20 µs in duration. A perfect 20 µs rectangular pulse stimulus cast into the frequency domain is a broad-band stimulus, that drops to a factor of ~0.7 at 25 kHz and to zero at 50 kHz, followed by side lobes. When cast into the frequency domain the click showed structure below 5 kHz, was nearly flat from ~ 5 to 20 kHz, and then sloped downward irregularly but contained significant energy up to ~40 kHz (Fig. 1.) The click responses were measured over 2 ms, thus 500 Hz is the resolution of the frequency-domain representation. For the click experiments, the acoustic stimuli and velocity responses were recorded and time-averaged (~500 averages) by a LeCroy oscilloscope sampling at 500 MHz, whose sweeps were triggered by the voltage pulses driving the speaker. The averaged responses were transferred to a computer for offline analysis using software written in MATLAB. The microphone was a Sokolich probe tube ultramicrophone. The velocity was measured with a laser interferometer, described later.

The multitone stimulus was a set of zwuis complexes, which deliver many frequencies at the same time without overlap of higher-order distortion products (Van der Heijden & Joris, 2003).

The middle ear is linear to very high sound level, (Huang et al., 2012) so this aspect of the zwuis is not as critical for middle ear measurements as for cochlear measurements, but it was useful for providing a well-controlled multi-tone level. An earbud was used as speaker and was driven by the TDT RX6 signal processor, attenuator PA5, and HB7 headphone buffer. The acoustic and velocity responses were recorded into the TDT RX6 and analyzed with MAT-LAB. Three different but overlapping zwuis complexes were used: 1) a Low Frequency probe with 20 tones between 200 Hz and 2 kHz (termed VLFZ for "very low frequency zwuis"), 2) a Medium Frequency probe with 40 tones between 400 Hz and 20 kHz (MFZ), and 3) Wide Frequency probe with 40 tones between 1500 Hz and 50 kHz (WFZ). As shown in Fig. 2, the distribution of frequencies in the WFZ becomes sparse above 45 kHz. All zwuis complexes were 5s in duration. A cosine envelope with a 0.5 ms on/off ramp was applied to the zwuis signal to reduce onset/offset transients. The voltage to the speaker was calibrated to produce equal sound level at each frequency. The frequencies in the zwuis stimulus were set so their periods fit evenly within the data collection window. An FFT was applied to the recorded time waveform of the response to find the frequency domain response. Generally, the zwuis probes were delivered at either 70 or 80dB SPL (per tone) to maximize the signal-to-noise, although some early series were conducted at 50 and 60dB SPL. (SPL is for "sound pressure level" where the stimulating pressure amplitude can be found using SPL = 20log(P/Pref), where Pref is 20µPa.) Spectra of each zwuis complex as delivered by the earbud speaker at 70dB and measured by the Sokolich ultramicrophone are in Fig. 2.

2.2. Animal preparation

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Columbia University. The gerbils were sedated with ketamine and anesthetized with pentobarbital (doses: 3 mg ketamine, 40 mg/kg pentobarbital). Subsequent doses of pentobarbital were given as indicated by the presence of a response to a toe-pinch. Buprenorphine was given for analgesia, dosed every six hours (dose: 0.2 mg/kg). The eyes were not treated with lubricant as these were acute non-survival surgeries. Body temperature was monitored and maintained with a rectal probe and thermostatic heating pad. The skull was affixed to a headholder. A tracheostomy was performed to maintain a patent airway. The pinna was removed and the tissue overlying the bulla was dissected and removed. The bony meatus overlying the umbo was cut back without entering the middle ear cavity, to improve the viewing angle to the umbo. A 27 gauge needle (outer diameter 0.4mm) was used to make a vent in the bulla to account for the lack of Eustachian tube function under anesthesia, which can



Fig. 2. Spectra of zwuis Complexes at 70dB as measured by the Sokolich ultramicrophone positioned just outside the ear canal, ~ 5 mm from the eardrum. Small deviations from 70 dB occur likely due to small shifts following the sound calibration (for example, around 19 kHz in the middle frequency zwuis). Results are presented as gain (velocity divided by simultaneously measured pressure), so these deviations will not affect the results.



Fig. 3. View of the Tensor Tympani Muscle with Electrode Placed. The stapedial artery courses between the anterior stapes crus (labeled) and the obscured posterior crus to its right. The TTM appears as a dome shape with a thin region at the top that contacts the manubrium (contact point is obscured by the TM in this view). The electrode contacts the plump dome of the TTM, and only that portion of the electrode is easily seen, due to microscope focus. A video recording of the muscle contraction in response to the pulse train is available in a supplemental file, and is useful for clarifying the 2-d image.

cause the middle ear space to become pressurized (Zheng et al., 1997). An opening in the bulla was made inferiorly and medially and expanded only as much as necessary to visualize the middle ear muscles. In the click set of experiments the stapedius was intact. In the zwuis set the stapedius tendon was sectioned near its insertion on the neck of the stapes. (Sectioning of the stapedius was included to ensure that incidental contraction of the stapedius was not contributing to the effects we measured but did not result in a significant change in the results.) Using a micromanipulator, a tungsten electrode was placed such that the tip touched the belly of the TTM, as shown in Fig. 3. A silver wire was placed in a muscle in the neck of the gerbil to provide the reference for the voltage stimulus.

2.3. Electrical stimulus

Nuttall's experiments in guinea pig (Nuttall, 1974) guided our choice of the electrical stimulus used to stimulate the TTM. The pulse train stimulus consisted of voltage pulses 100 µs in duration, delivered at a rate of 100 Hz. The voltage stimulus caused a brisk, visible contraction of the muscle and displacement of the eardrum, which was monitored via the video camera that is part of the Polytec laser vibrometer. The sound stimulus was delivered after this rapid deflection of the eardrum was observed. The electrical stimulus was discontinued as soon as the sound stimulus was over. Two minutes were allowed between each stimulation of the muscle. For the click experiments the voltage pulse size was typ-



Fig. 4. A. Velocity response measured at the umbo to click stimulus from Fig. 4. B. Frequency domain representation. The reduction in energy in the click at high and low frequencies and noise level of the velocity limited the findings on differences between contracted and non-contracted states to the frequency band between 2 and 35 kHz (when using the click stimulus).

ically 5V, based on visualization of muscle contraction when setting up the experiment. With the zwuis experiments, we tested various amplitudes and found that the visual intensity of the contraction saturated around 4V, and this was corroborated by the velocity response data. Thus, a 4V pulse size was used in this set. A solid but slightly submaximal contraction was desirable since the muscle was stimulated dozens of times in each experiment over a period of hours. The data indicate the contraction produced by the stimulus remained highly consistent run-to-run and over the course of the experiment.

2.4. Measurements

The head was oriented so the beam of the laser interferometer was initially focused on the umbo. A laser Doppler vibrometer (LDV) was used to record the velocity responses of the umbo and other locations along the ossicular chain (Polytec LDV OFV-534, and VD-06 decoder). The LDV's Helium-Neon laser was focused on the preparation with a 5x Mitutoyo lens with 33.5 mm focusing distance and focused spot size of diameter ~ 3 μ m.

For the click set of experiments the bulla was open, which reduced the umbo response at frequencies below ~ 5 kHz. In order to probe this lower frequency region more accurately, in the second, zwuis set of experiments, after placing the electrode, surgical Ethicon bone wax was placed around the electrode to seal the opening. Opening the bulla is known to affect middle ear transmission, and these effects have been studied in gerbils. When using a sound source that is sealed within the ear canal, progressively opening the bulla decreased the stiffness of the middle ear which increased low-frequency responses, shifted down the main middle ear resonance, and produced an antiresonance that increased in frequency with the extent of bulla opening (Maftoon et al., 2014). In our setup, in which the sound was delivered open-field, our priority was to prepare an acoustically intact bulla that would not allow an accessory route for sound waves to stimulate the eardrum from the back. We investigated the efficacy of closing the bulla opening with bone wax by measuring sound-stimulated umbo motion with the bulla 1) vented (0.4mm diameter) but otherwise intact, 2) opened as necessary for placement of the electrode, and 3) once that opening was closed with wax. Our results, shown in the section below, indicate that bone wax closure successfully reversed the effects of opening the bulla on umbo motion. The bulla was open in the click experiments as the closure technique had not yet been developed during the first series of experiments. This difference will be discussed later with a comparison of the frequencystructure of the data from the click and multitone experiments.

The velocity response at the umbo was measured in a series of repeating runs with a single stimulus (click or zwuis) with the TTM stimulated every other run. The number of runs in a series is indicated in the reported data. In the zwuis experiments, following a series of umbo measurements the pars flaccida (PF) was removed to allow the laser to be focused on the LPM, LPI and PLP (lateral



Fig. 5. Diagram of the four sites at which velocity measurements were taken with the LDV. Lateral process of the malleus (LPM), Long process of the incus (LPI), Plate of the lenticular process of the incus (PLP).

process of the malleus, long process of the incus and the plate of the lenticular process). The method of opening the pars flaccida to measure along the ossicular chain has been described in detail in earlier reports from our group (de La Rochefoucauld et al., 2010). Fig. 5 diagrams these measurement sites. For LPI and PLP measurements, effort was made to orient the laser to the axis of the piston motion of the stapes.

3. Results

3.1. Click experiments

In keeping with the chronology of the experiments, the time domain click stimulus results are presented first. Fig. 1A shows the acoustic click in the time domain, and Fig. 4A shows the corresponding umbo velocity response – these are baseline measurements, without TTM stimulation. In Fig. 1B and Fig. 4B, the time domain acoustic and motion measurements were cast into the frequency domain. The acoustic frequency response was reasonably flat and within a factor of ~10 from ~2 to 28 kHz, beyond which it dropped off. The velocity response was flat within a factor of ~10 from ~2 to 30 kHz, beyond which it also dropped off. In or-



Fig. 6. Velocity response measured at the umbo to the click acoustic stimulus. A and B. Uncontracted and contracted responses from expt. 4-9-19; C and D. Uncontracted and contracted responses from expt. 4-17-19.



Fig. 7. A-D show the velocity responses from each experiment as averages. E-H. Averaged uncontracted and contracted responses from four experiments. E-H: same as A-D but emphasizing the first 0.2 ms of the response. (D and H are offset in time slightly compared to other experiments due to an inadvertent triggering shift in the oscilloscope.)

der to estimate the noise level in the click data, we performed a Fourier transform of the second half of the velocity time response (from 1 to 2 ms) where the response had largely died out. The upper-bound noise level of the velocity response varied depending on the reflectivity but was generally ~ .003 mm/s. In the frequency-domain version of the click responses, below ~ 2 kHz and above ~ 35 kHz the umbo velocity responses were close enough to this noise level that differences between the contracted and non-contracted state were not reproducible. In Fig. 4 B and C and responses above 35 kHz and below 2 kHz are grayed over to convey that in these frequency regions, the results on the effect of contraction were not robust, and when frequency-domain versions of the click responses are presented in Fig. 14, results are shown from 2 - 35 kHz. Fig. 4C, the ratio of umbo velocity to sound pressure frequency responses illustrates the broadband response of the middle ear, where from 2 to 35 kHz, the transfer function is reasonably flat at ~ 1-2 (mm/s)/Pa.

Fig. 6 summarizes the time-domain umbo velocity results from two experiments of the click series. Fig. 6A-B are runs 14 to 30 from Expt. 4-9-19 with the uncontracted cases in A and the contracted cases in B. Fig. 6 C-D are runs 19 to 44 from Expt. 4-17-19 with the uncontracted cases in C and the contracted cases in D. TTM contraction was alternated between runs. These repeated measurements convey the subtle but repeatable changes observed upon contraction of the TTM. Fig. 7 A-D are averages of data like

that in Fig. 6 A-D - each panel reports a different experiment. In Fig. 7 E-H the initial segments of the data from Fig. 7 A-D are expanded to explore contraction-induced size and timing changes in the main peak and valley of the response. In all data sets, repeatable changes were observed with TTM contraction, but consistency between preparations was not observed. When the TTM was contracted, the size of the first positive peak in the umbo velocity response was reduced slightly in Fig. 7 E and G, and the size of the first negative peak was increased slightly in Fig 7 E, F and H. In Fig. 7B, the ringing that followed the initial response was a lower frequency in the contracted versus relaxed condition and in Fig. 7D, the opposite was true. changes in delay were very small. Evaluated quantitatively by considering the difference in the times the response crossed from positive to negative at the end of the positive peak, the following times were found: Expt 4-9-19: 0.7 us (contracted condition was delayed relative to uncontracted by this amount), Expt 4-17-19: 0.4 µs, Expt 4-30-19: 0.8 µs, Expt 5-6-19: -1 µs. These differences are at most a microsecond, are not consistent in direction, and deemed insignificant.

3.2. Zwuis experiments

The zwuis tone complexes produced velocity responses with clear spectral peaks above the background. Fig. 8 shows the spectra



Fig. 8. Spectra of the velocity response at the umbo from expt. 10-16-20. These velocity reponses were measured at the umbo in response to VLFZ and MFZ at 80dB SPL and WFZ at 70dB SPL.



Fig. 9. A bone wax closure of the surgical opening in the bulla reversed the changes in umbo motion produced by the opening.

of the velocity response at the umbo for each zwuis from experiment 10-16-20.

We investigated the efficacy of our use of bone wax to close the bulla for the zwuis experiments. Umbo motion in response to a zwuis tone complex was measured three times each with the bulla vented, opened, and once the electrode was placed and the surgical opening occluded with bone wax, but with the vent remaining patent.

Fig. 9 shows the results of once such series, which were repeated in multiple animals with consistent results. The data show that opening the bulla produces substantial changes in umbo motion and that these changes are reversed when the opening is occluded with bone wax. (Recall that sound delivery is open field, and the open bulla allows for sound to stimulate the back of the eardrum; closing the bulla eliminated this path.)

We investigated the effect of varied levels of electrical stimulation of the TTM by varying the amplitude of the voltage pulse train stimulus. Our subjective observations that the visible intensity of the TTM contraction saturated at an amplitude of 4V was corroborated by our measurements of the gain at the umbo with various stimulus amplitude. These results, presented in Fig. 10, show that a 4V stimulus produced a near maximal contraction of the muscle vis-à-vis its effects on umbo motion.

Although velocity measurements of the umbo and ossicles are less likely to be directly (artifactually) affected by an electrical stimulus than the cochlear microphonic measured by Nuttall, we controlled for this possibility by testing the stimulus with the electrode removed from the muscle by about 1mm. We also measured

Umbo Motion with Different Stimulus Amplitudes (7-30-20)



Fig. 10. Contraction-induced changes in umbo motion varied with the amplitude of the electrical stimulus delivered to the TTM. A 4V stimulus amplitude appears to produce near-maximal changes in the umbo motion. There are relatively slight differences between 4V, 6V, and 8V above 20kHz, but these differences do not correlate with voltage amplitude.

the effect of the muscle contraction after the tendon had been cut. We investigated whether the physical deflection of the pars tensa might create an artifact by displacing the point on the umbo where the interferometer laser was focused. Fig. 11 presents these data, which show that the stimulus had no effect on umbo motion when the electrode was not touching the muscle or when the muscle was not attached to the malleus.

3.3. Gain changes with TTM contraction

We present results organized by zwuis frequency range and site measured. We investigated the effect of the TTM contraction at multiple points along the middle ear transmission chain: umbo, lateral process of the malleus (LPM), long process of the incus (LPI), plate of the lentiform process of the incus (PLP). (For the LPI and PLP the pars flaccida was removed so the middle ear was open during these measurements.) The number of alternating runs in each series ranged from 4 to 10. These series produced repeatable and consistent velocity data from run to run. Fig. 12 is an example of all of the velocity response data from one experiment in which each zwuis probe was tested at each site of measurement on the ossicular chain.

In the uncontracted state, the amplitude of the gain varied with frequency and point measured. Gain was greatest at the umbo and ranged between 0.01 and 1 mm/s/Pa, comparable with earlier reports (de La Rochefoucauld et al., 2010). In general, gain decreased



Fig. 11. Some control results from experiment 11-3-20. The stimulus only produced changes in umbo motion when touching TTM and with intact TTM. For the series presented in A & B, the pars flaccida had been removed, because LPI and PLP measurements had been taken earlier in the experiment. A shows an alternating series with the electrode removed from the TTM by ~3mm. B shows an alternating series once the TTM tendon was cut and the electrode was replaced on the muscle. Under each of these conditions, the stimulus failed to produce changes in umbo motion. C shows the umbo motion with the WRZ with alternating a slight repositioning of the LDV focus point to simulate the slight deflection produced by TTM contraction. Repositioning did not produce the changes in umbo motion seen with TTM contraction.

downstream with gain at the LPM slightly less than that at the umbo. The decrease in gain amplitude downstream of the LPM was greater, but gain at the LPI and PLP were very similar to each other. The velocity response data measured at the PLP, as a proxy for the stapes footplate motion, are comparable to those reported by other investigators (Overstreet & Ruggero, 2002)(Ravicz et al., 2008). The frequency structure was similar across the points measured - fairly flat above 5kHz. The LPI and PLP measurements were made with the pars flaccida removed for visualization, and in those cases the responses below 5 kHz were attenuated, and a mild peak was present at ~ 5kHz. TTM contraction mostly reduced middle ear vibrations but did produce an increase in response from ~ 4-8 kHz (umbo and malleus) and 6-8 kHz (LPI and PLP). The effect produced by stimulating the muscle was consistent in the overlapping frequencies of the different multitone stimuli. At the highest frequencies probed, the velocity responses were less repeatable, particularly at the PLP and LPI, which may be a result of poorer frequency resolution of the zwuis complex above 45 kHz and spatial non-uniformity of the sound field. Additionally, ossicular motion becomes more complex at higher frequencies, which could produce variability in incus vibrations (de La Rochefoucauld et al., 2010)(Decraemer et al., 2014).

Each of the alternating series shown in Fig. 12 can be condensed and represented as an average ratio of the gains of the ear with versus without stimulating the TTM. Each gain ratio was calculated by dividing the averaged gain of the stimulated runs by the averaged gain of the unstimulated runs for a given alternating series. The data from experiment 10-16-20 in Fig. 12 and other multitone experiments will be presented as gain ratios and average phase differences, averaged within individual preparations. Not all stimuli were used in all experiments, for example the VLFZ stimulus set was added after several experiments had been done.

3.4. Frequency structure at the umbo

Contraction of the TTM produced effects on umbo motion that were repeatable and consistent across experiments. Fig. 13 shows the average gain ratio and average phase difference at the umbo using each zwuis probe from each experiment in which these measurements were taken.

The data from the VLFZ and MFZ probes show that the TTM attenuates umbo motion by about 2-5 times at the lowest frequencies. The data from the WFZ reveals that the muscle attenuates frequencies above 35 kHz by a comparable degree. In general, the umbo gain ratios were consistent across experiments, but there were certain frequency ranges in which the TTM's effect ap-

peared particularly conserved. For example, the frequency above which the muscle begins to enhance umbo motion (i.e., when the gain ratio crosses 0) was always very close to 4 kHz, while the maximally enhanced frequency varied from about 5 kHz to 7 kHz. The frequency-structure where there was overlap between zwuis multi-tones was consistent. The effects of TTM contraction observed with the MFZ stimulus, including 40 frequencies spanning 400 Hz to 20 kHz, were consistent where this probe overlapped with the VLFZ stimulus. The phase difference (contracted - uncontracted) showed a lead that generally grew to almost 0.2 cycles at ~ 4 kHz, and then dropped, passing through zero at ~ 6 kHz and then meandering through two mild and broad variations from 6 to 50 kHz.

As noted above, two sound stimuli were used in these experiments, a click and a zwuis. The time-domain data using the click stimulus have already been described. The zwuis stimuli were intended to probe the frequency-structure of TTM contraction, but it is also possible to present the gain ratio of the frequency-domain representation of the click experiment results.

Fig. 14 shows the gain ratio curves from the click experiments in black compared with the gain ratio curves from the Wide Frequency zwuis series at the umbo in gray. As noted above, the click stimulus responses were robust only from ~ 2 to 35 kHz. Within this range the frequency-structure of TTM effect across the click and zwuis stimulus types was consistent with the exception of some differences below 5 kHz, where structure is seen in two of the four click results. The click measurements were done with an open bulla and, as Fig. 9 showed, opening the bulla could introduce a notch and a peak into the umbo response at frequencies below 5 kHz. Contraction-induced changes in a region of a notch or peak can introduce large changes in the gain ratio. Investigating the unaveraged data (not shown) corroborates this explanation as the basis for the increased structure in two of the click experiments.

3.5. LPI and PLP

The behavior of the middle ear downstream of the umbo was also of interest, so velocity responses were measured at the LPI and PLP. The PLP is part of the incus but is tightly coupled to the head of the stapes. The angle of measurement with the vibrometer through the opened pars flaccida closely approximated the pistonaxis of the stapes. As such, the PLP velocity response served as a proxy for footplate motion and, by extension, middle ear output.

Measuring velocity response at the LPI and PLP was complicated by the need to open the PF to train the laser on sites within



Fig. 12. Velocity response data, shown as gain, from one experiment (10-16-20), measured at the umbo, lateral process of the malleus (LPM), long process of the incus (LPI), and plate of the lenticular process of the incus (PLP) with each zwuis probe in alternating series (Very Low Frequency Zwuis 'VLFZ', Medium Frequency Zwuis 'MFZ', Wide Frequency Zwuis 'WFZ'). The red represents runs in which the TTM was contracted, the blues those in which the TTM was not contracted. The shadows represent the mean of each group +/- one standard deviation. N represents the total number of runs in each alternating series.

the middle ear. This was a delicate step in the experiment and not always successful. Additionally, the pars flaccida is an acoustically significant structure, particularly at low frequencies. Experiments have suggested that that it may serve as a shunt around the pars tensa for sound, reducing the effective input to the middle ear (Teoh et al., 1997). Opening the PF should enhance this shunting effect. The effects of eardrum perforations on sound transmission have been studied in human ears as well (Voss et al., 2001). We found that opening the pars flaccida did attenuate umbo motion at low frequencies, which made the use of the VLFZ probe difficult at the LPI and PLP. We considered that the absence of the PF might interact with the contraction of the TTM. To investigate this question, we compare the gain ratios produced by TTM contraction at the umbo before and after opening the PF.

Fig. 15 shows that, with an opened PF, TTM contraction produced an area of relative enhancement around 3 kHz. The presence of a similar enhancement in the gain ratios at the LPI and PLP – discussed below – is likely the result of the open state of the pars flaccida during the measurements.

Fig. 16 shows that the effect of TTM contraction at the LPI at low frequencies was variable, sometimes enhancing and at others attenuating. Referring back to Fig. 12, from 4-8 kHz there is a mild response peak that shifts between the contracted and uncontracted

states and gives rise to the somewhat erratic gain ratio in that frequency region in Fig. 16A. At frequencies below 4 kHz, the complications of PF removal (Fig. 15) might have made the results less robust. Above 10 kHz, the gain ratio remained below 1 in most experiments, with the exception of expt. 7-30-20 in which the gain ratio briefly crossed 1 around 24 kHz. At higher frequencies, the attenuation became larger as shown in Fig. 16 A and B. The phase difference generally showed a lead to ~ 7 kHz, followed by a mild lag, and then was close to zero to ~ 30 kHz where it again developed a broad mild lag.

Measurement at the PLP was technically more difficult than at the LPI, requiring repositioning of the gerbil in relation to the LDV and consequently removal and replacement of the electrode.

The PLP was successfully probed three times. The gain ratio curves across experiments were more consistent than than those measured at the LPI, but the frequency structure at the LPI and PLP was overall similar. This apparent relative consistency of the PLP data could be incidental because the PLP was measured fewer times compared to the LPI. Compared to the umbo, the TTM's attenuating effect tends to predominate. The area of enhancement around 3 kHz may be the result of the opening the PF – a similar enhancement region was observed (Fig. 15) in the umbo motion data when the PF was opened.



Fig. 13. TTM gain ratios and phase differences at the umbo with each multitone stimulus. The parenthetical numbers in the legends represent the number of runs in each alternating series. A & D VLFZ gain ratios and phase differences spanning 0.2 to 2 kHz. B & E MFZ gain ratios and phase differences spanning 0.4 to 20 kHz. C & F WFZ gain ratios and phase differences, spanning 1.5 - 50 kHz.



Gain Ratio: WFZ and Click Stimulus at the Umbo

Fig. 14. Frequency structure of TTM effect on umbo motion was consistent across experiments using Click and zwuis multitone stimuli.

4. Discussion

In the time domain responses, TTM-induced changes were subtle but repeatable. A significant finding of the time-domain measurements was that the contraction of the TTM did not change the delay of sound transmission by the eardrum, as measured at the umbo. This finding is at odds with the idea that a role of the TTM is to introduce a delay to enhance timing-based lateralization cues (Cho et al., 2019). This role might be present in species other than gerbil. By casting the click domain results into the frequency domain, frequency dependent effects were observed and as in the time-domain were subtle but repeatable. They were consistent with the frequency domain results observed with the zwuis multitone stimulus (Fig. 14), and we emphasize the multi-





Fig. 15. The condition of the pars flaccida interacted with the contraction of the TTM. Notably, this produced a second area of relative enhancement around 3 kHz, where the solid (PF-) curves diverge from the dashed (PF+) curves.

tone stimuli when discussing frequency domain results, because they covered a wider frequency range.

Contraction of the TTM attenuated motion at low and high frequencies. However, it produced a relative enhancement of transmission in a frequency band around ~6 kHz. Our voltage-elicited TTM contraction was based on that of the guinea pig study of Nuttall (Nuttall, 1974), and that study's analysis is useful to understanding the band of enhancement, and low frequency attenuation. In the guinea pig study, the effect of stapedius and TTM contraction on sound-induced cochlear microphonic (CM) was measured over a frequency range of 70 Hz - 6 kHz. The data were presented as magnitude and phase change, as in our gain ratio results (Fig. 13). In the guinea pig, contraction of the TTM produced attenuation of ~20dB for frequencies below 1 kHz, that then shifted to a mild peak in which the contraction caused positive gain from ~ 2 to 4 kHz, peaking at ~5dB. With contraction the CM phase underwent an increasing phase lead to 1.5 kHz, where it peaked at ~ 1/4 cycle, then the lead decreased back to zero. Qualitatively, our findings share similarities with the guinea pig study, with similar features shifted to slightly higher frequencies. Contraction of the TTM caused umbo motion attenuation of ~ 10 dB at frequencies from 200 Hz to 1 kHz (Fig. 13A), and the attenuation was similar or slightly larger at the PLP (Fig. 17A). The gain ratio became positive from ~ 4 to 10 kHz with a mild peak at ~5-6 kHz of a factor of ~2 (6 dB) when measured at the umbo (Fig. 13C). The phase difference was a lead of up to almost 0.2 cycle, with the lead persisting to ~ 6 kHz. From 6 - 50 kHz the phase difference meandered around zero through two broad variations, and the gain ratio also meandered through two peaks and valleys although above 30 kHz the gain ratio was always less than one - the TTM contraction caused attenuation.



Fig. 16. TTM gain ratios and phase differences at the LPI with MFZ and WFZ multitone stimuli. The parenthetical numbers in the legends represent the number of runs in each alternating series. A & C. MFZ gain ratios and phase differences spanning 0.4 to 20 kHz. B & D. WFZ gain ratios and phase differences, spanning 1.5 - 50 kHz.



Fig. 17. TTM gain ratios and phase differences at the PLP with MFZ and WFZ multitone stimuli. The parenthetical numbers in the legends represent the number of runs in each alternating series. A & C. MFZ gain ratios and phase differences spanning 0.4 to 20 kHz. B & D. WFZ gain ratios and phase differences, spanning 1.5 - 50 kHz.

In the relatively narrow frequency range of Nuttall's guinea pig study, the frequency-structure of the response to TTM contraction could be quantitatively fit by a second order lumped element model of middle ear transmission composed of a spring-massresistance in series. In the model, contraction of the TTM produced an increase in stiffness and decrease in resistance. The increased stiffness attenuated the low frequency responses and shifted an overdamped spring-mass resonance to a slightly higher frequency, causing increased gain over a narrow range. This resonanceshifting effect has been observed in other experimental investigations of the TTM (Møller, 1965). The increased stiffness extended the low frequency stiffness-dominated region in which velocity leads pressure to higher frequencies, causing a phase lead of the contracted relative to the uncontracted case. A simple illustration is given in Fig. 18. This model and explanation would work for frequencies through the first peak of our umbo gain ratio, ~ 10 kHz (compare data in Fig. 13 B and E) but the lumped element model is too simple to explain the changes we observed at higher frequencies.

Above a few kilohertz, the eardrum and ossicles exhibit complex vibrations, and the eardrum behaves as a distributed wave system that allows for transmission of high frequency sounds (Funnell et al., 1987)(Puria & Allen, 1998)(de La Rochefoucauld & Olson, 2010)(Milazzo et al., 2017). Several computational models have been developed to explore middle ear operation through a wide frequency range. One published model – a combination of rigid bodies and finite elements – was designed to consider the effects of the TM's angle, conical shape, and radial fibers (Fay et al., 2006). This model found that increasing the depth of the TM's shape – as the medially directed load the TTM places on the manubrium does – would cause a decrease in gain at frequencies up to ~ 2 kHz, and an increase from 2 to 10 kHz, followed by more random variations in gain. The deepening of the TM's conical shape also caused a phase lead up to ~ 4 kHz that was ~ 0.15 cycle at 2 kHz. While the lumped-element resonant circuit model is useful for developing basic understanding of the effect of TTM contraction, in particular the mild peak in gain ratio that follows the predictable reduction at low frequencies, a distributed model like that of (Fay et al., 2006) is likely to be required to understand the full effect of TTM contraction observed in our study.

While contraction-induced changes in gain were described above as occurring over fairly broad frequency ranges, relatively sharp frequency variations were also observed. When they appeared at the lowest and highest frequencies, these variations were sometimes due to noise, because at these frequencies the vibrations could be relatively small and were further attenuated by TTM contraction. For example, in Fig. 12 the contraction-induced trends were highly repeatable, but all of the sharp variations were not repeatable, particularly below 1 kHz, and above 45 kHz at the LPI and PLP. However, many sharp variations were robust. For example, the expt. 10-16 gain ratio in Fig. 16A showed a sharp notch at 1 kHz and wiggles at 3 and 5 kHz. The individual run data condensed in that ratio are shown in Fig. 12H; through three cycles of contraction and relaxation (six runs), the sharp frequency-



Fig. 18. Spring-mass-resistance lumped element system can produce the observed gain ratio and phase difference by modeling the TTM contraction as increasing the stiffness and decreasing the resistance. A and B are magnitude and phase of the second order system, in the contracted and uncontacted states. C and D show the ratio of magnitudes and phase differences. Compare A to Fig. 12B results, compare C&D to Fig. 13 B&E.



Fig. 19. Example in which contraction-induced changes introduced repeatable fine structure in the umbo vibration. (Blues = uncontracted; reds = contracted). The structure that appeared with contraction at 5-6 kHz in the intact state persisted following removal of the PF, and was a robust feature of contraction in this preparation (10-5).

dependent variations were repeatable. Another example is Fig. 13B, where a sharp valley at 5-6 kHz is observed in data set 10-5. This was a robust and repeated variation in the basic data, even after PF removal, as shown in Fig. 19. Sharp frequency-dependent contraction-induced variations are not predicted by the simple filtering that was described in Fig. 18 but are consistent with a view of middle ear mechanics as a sum of mistuned resonances (Funnell et al., 1987)(Milazzo et al., 2017).

Many questions remain about the behavior of the TTM in nature. One is the question of whether the muscle acts uni-

laterally or bilaterally. The stapedius is thought to act bilaterally, although experiments using the acoustic reflex to produce contraction have found differences in intensity of contraction between the ipsilateral and contralateral stapedius muscles (Møller & Moore, 2001). A sufficient sound stimulus produces contralateral contraction of the TTM; however, the contralateral contraction has been observed to be considerably weaker than the ipsilateral contraction (Møller, 1965). It appears likely that the action of the TTM is not perfectly symmetrical.

Although the time-domain data from our click experiments do not support the idea that TTM contributes to lateralization cues via interaural delay differences, intensity differentials are also powerful lateralization cues (Erulkar, 1972). If the TTM contracts asymmetrically, it likely modulates these interaural pressure differentials, possibly playing a role in localization or attention. A recent report found that in humans and monkeys saccadic ocular movements produce movements of the eardrums even in the absence of auditory stimuli (Gruters et al., 2018). These oscillating movements of the eardrums, possibly the products of MEM contraction, were directionally consistent with directional eye movements. Indeed, further investigation has shown that eye movement-related eardrum oscillations (EMREOs) are a function of eye movement and absolute eye position (Murphy et al., 2020). The mechanism of this interaction between sight and hearing has not been fully elucidated, however, should MEMs be found responsible for these EMREOs, the muscles would appear to have a spatial orientation function of some kind.

The zwuis experiments were designed to measure the frequency structure of TTM effects on middle ear transmission. The frequency-domain data revealed repeatable patterns of middle ear transmission modulation, which at the lower frequencies can be explained by an increased stiffness. Aside from a range of enhancement at the frequency of shifted overdamped spring-mass resonance (about 6 kHz in our gerbils), the TTM predominantly attenuated umbo and ossicle vibrations. It was not an expected finding that the TTM attenuated transmission at higher frequencies by the same degree it attenuated low frequencies. It is notable that the frequency range with the least attenuation was about 20 kHz to 30 kHz. In this range, the muscle's effect was close to neutral on average. Gerbil vocalizations are tonally and temporally complex, with 80% at frequencies above 20 kHz (Nakayama & Riquimaroux, 2017). Vocalizations in the 20 kHz to 30 kHz range are biologically important and include mating and greeting calls. One group of investigators found that cochlear microphonic responses at ultrasonic frequencies were enhanced by 2-5 dB when gerbils were paired with a companion gerbil (Nakayama & Riquimaroux, 2017). Cochlear responses below 20 kHz did not display this companionenhancement effect. At the umbo our gain ratio show a mild peak from 20-30 kHz that produced a gain ratio slightly greater than 1 (mild enhancement) (Fig. 13C). At the LPI and PLP, the TTM produced attenuation above 10 kHz but the attenuation was smaller in the 20-30 kHz range. One can imagine a coordination of middle ear modulation and cochlear efferents that might be able to produce the observed enhanced response in the cochlear microphonic.

While the TTM may help gerbils hear the calls of their mates and companions, hearing is a specialized sense; it is possible that the muscle may have a function that varies across species. Middle ear anatomy varies widely among terrestrial animals to accommodate specific hearing needs (Rosowski, 2003). Within Rodentia, middle ear morphology is particularly variable (Mason, 2015). Ossicular shape, arrangement of connective tissues, cavity size and shape all vary considerably between species. The histochemistry of the middle-ear muscles is also variable, and the TTM, in particular, demonstrates wide inter-species variation in cellular structure and histologic development (Veggetti et al., 1982) (Mascarello et al., 1983). Interspecific variation in the TTM provides both a window and veil for investigations into its function. Structural, cellular, behavioral, and acoustical variation in the muscle placed in the context of a particular animal's auditory needs may enhance our understanding; however, it also limits the inferences we may draw from animal models to inform human hearing.

It will be important to correlate the TTM-mediated effects measured here and previously with the actual incidence of TTM contraction in the natural life of a gerbil, other important auditory model animals, and humans. More direct observations of when the muscle contracts in alert gerbils are needed. We considered a few approaches to this investigation. Fortunately, the TTM produces a visually obviously movement of the eardrum. This was true for our electrically stimulated contraction, and other investigations, such as those by Funnell (Funnell, 1972) and in our own guinea pig work, found that spontaneous contractions of the muscle were plainly visible in the motion of the eardrum. The approaches we consider take advantage of this fact. Fine endoscopes are widely commercially available and may allow for direct visualization of the eardrum. The anatomy of the bony meatus in gerbil, however, could complicate the use of these devices in intact ears. Miniature contact-lens scleral coils have been used to measure ocular motions in mice (Kaneko et al., 2010). Such technology could be applied to the detection of movement of the eardrum relative to the head. Alternatively, we considered using a small mirror placed on the umbo and light-sensor device glued to the ear canal, in which TTM contraction moves the reflected light on or off the sensor. Investigations such as these will provide important context to the physiological findings we have reported here.

Declaration of Competing Interest

The authors have no competing interests to declare.

Acknowledgements

We thank Elika Fallah and C. Elliott Strimbu for experimental guidance and assistance and the two Hearing Research reviewers, John Rosowski and a second reviewer who remained anonymous, for improving the paper.

Funding sources

This work was funded by NIH/NIDCD grant number DC015362, and by the Emil Capita Foundation.

Author statement

The contributions of each author are briefly described below: • Elizabeth Olson supervised theexperiments.

• Mohamed Diop performed the initial set of experiments which used a click stimulus.

• Liam Gallagher performed the subsequent set of experiments which used zwuis multitone stimuli.

• Elizabeth Olson and Liam Gallagher co-wrote the manuscript, with input from Mohamed Diop.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.heares.2021.108231.

References

- Angeli, R.D., Lise, M., Tabajara, C.C., Maffacioli, T.B., 2013. Voluntary contraction of the tensor tympani muscle and its audiometric effects. J. Laryngol. Otol. 127 (12), 1235–1237. doi:10.1017/S0022215113003149.
- Avan, P., Loth, D., Menguy, C., Teyssou, M., 1992. Hypothetical roles of middle ear muscles in the guinea-pig. Hear. Res. 59 (1), 59–69. doi:10.1016/0378-5955(92) 90102-S.
- Bance, M., Makki, F.M., Garland, P., Alian, W.A., Van Wijhe, R.G., Savage, J., 2013. Effects of tensor tympani muscle contraction on the middle ear and markers of a contracted muscle. Laryngoscope 123 (4), 1021–1027. doi:10.1002/lary.23711.
- Borg, E., 1972a. On the change in the acoustic impedance of the ear as a measure of middle ear muscle reflex activity. Acta Oto-Laryngol. 74 (1-6), 163-171. doi:10. 3109/00016487209128437.
- Borg, E., 1972b. Regulation of middle ear sound transmission in the nonanesthetized rabbit. Acta Physiol. Scand. 86 (2), 175–190. doi:10.1111/j.1748-1716. 1972.tb05324.x.

- Borg, E., Counter, S.A., Rösler, G., 1984. Theories of middle-ear muscle function. In: The Acoustic Reflex. Academic Press, Inc, pp. 63–99. doi:10.1016/ b978-0-12-643450-7.50008-1.
- Carmel, P.W., Starr, A., 1964. Non-acoustic factors influencing activity of middle ear muscles in waking cats. Nature 202, 195–196. doi:10.1038/202195a0.
- Carney, L.H., Sarkar, S., Abrams, K.S., Idrobo, F., 2011. Sound-localization ability of the Mongolian gerbil (Meriones unguiculatus) in a task with a simplified response map. Hear. Res. doi:10.1016/j.heares.2010.12.006.
- Cho, N., Puria, S., Ravicz, M.E., 2019. Human tympanic-membrane shape and middle-ear delay changes due to mechanically pulling the tensor-tympani muscle. In: Proceedings of the Midwinter Meeting of the Association for Research in Otolaryngology.
- de La Rochefoucauld, O., Kachroo, P., Olson, E.S., 2010. Ossicular motion related to middle ear transmission delay in gerbil. Hear. Res. 270 (1–2), 158–172. doi:10. 1016/j.heares.2010.07.010.
- de La Rochefoucauld, O., Olson, E.S., 2010. A sum of simple and complex motions on the eardrum and manubrium in gerbil. Hear. Res. 263 (1-2), 9-15. doi:10.1016/ j.heares.2009.10.014.
- Decraemer, W.F., de La Rochefoucauld, O., Funnell, W.R.J., Olson, E.S. 2014. Threedimensional vibration of the malleus and incus in the living gerbil. JARO - J. Assoc. Res. Otolaryngol. 15 (4), 483–510. doi:10.1007/s10162-014-0452-1.
- Djupesland, G., 1964. Middle ear muscle reflexes elicited by acoustic and nonacoustic stimulation. Acta Oto-Laryngol. 188, 287. doi:10.3109/00016486409134578.
- Dong, W., Olson, E.S., 2013. Detection of cochlear amplification and its activation. Biophys. J. 105 (4), 1067–1078. doi:10.1016/j.bpj.2013.06.049.
- Erulkar, S.D., 1972. Comparative aspects of spatial localization of sound. Physiol. Rev. 52 (1), 238–346. doi:10.1152/physrev.1972.52.1.237.
- Fay, J. P., Puria, S., & Steele, C. R. (2006). The discordant eardrum. Proceedings of the National Academy of Sciences of the United States of America, 103(52), 19743– 19748. doi:10.1073/pnas.0603898104.
- Funnell, W.R.J, 1972. Middle-ear impedance and effects of middle-ear muscles MS Thesis. McGill University.
- Funnell, W.R.J., Decraemer, W.F., Khanna, S.M, 1987. On the damped frequency response of a finite-element model of the cat eardrum. J. Acoust. Soc. Am. 81 (6), 1851–1859. doi:10.1121/1.394749.
- Galambos, R., Rupert, A., 1959. Action of the middle ear muscles in normal cats. J. Acoust. Soc. Am. 31 (3), 349–355. doi:10.1121/1.1907723.
- Gelfand, S.A., 1998. Hearing: An Introduction to Psychological and Physiological Acoustics, Third Edition. CRC Press.
- Gelfand, S.A., 2009. Hearing: An Introduction to Psychological and Physiological Acoustics, Fifth Edition. CRC Press.
- Gruters, K.G., Murphy, D.L.K., Jenson, C.D., Smith, D.W., Shera, C.A., Groh, J.M, 2018. The eardrums move when the eyes move: a multisensory effect on the mechanics of hearing. Proc. Natl. Acad. Sci. USA 115 (6), E1309–E1318. doi:10.1073/pnas. 1717948115.
- Gyo, K., Goode, R.L., 1987. Effects of middle ear pressure changes on umbo vibration. Auris Nasus Larynx 14 (3), 131–137. doi:10.1016/S0385-8146(87)80013-5.
- Honjo, I., Ushiro, K., Haji, T., Nozoe, T., Matsui, H., 1983. Role of the tensor tympani muscle in eustachian tube function. Acta Oto-Laryngol. 95 (3–4), 329. doi:10. 3109/00016488309130950, 329.
- Huang, S., Dong, W., Olson, E.S., 2012. Subharmonic distortion in ear canal pressure and intracochlear pressure and motion. JARO - J. Assoc. Res. Otolaryngol. 13 (4), 461–471. doi:10.1007/s10162-012-0326-3.
- Kaneko, C.R.S., Rosenfeld, S., Fontaine, E., Markov, A., Phillips, J.O., Yarno, J, 2010. A preformed scleral search coil for measuring mouse eye movements. J. Neurosci. Methods 193 (1), 126–131. doi:10.1016/j.jneumeth.2010.08.023.
- Lenarz, T., 2002. The use of gerbils (meriones unguiculatus) as an animal-model in ontogenetic cochlear-implant-research. Otol. Neurotol. 48 (3), 209–214. doi:10. 1097/00129492-200211000-00042.
- Maftoon, N., Funnell, W.R.J., Daniel, S.J., Decraemer, W.F. 2014. Effect of opening middle-ear cavity on vibrations of gerbil tympanic membrane. JARO - J. Assoc. Res. Otolaryngol. 15 (3), 319–334. doi:10.1007/s10162-014-0442-3.
- Mascarello, F., Veggetti, A., Carpene, E., Rowlerson, A., 1983. An immunohistochemical study of the middle ear muscles of some carnivores and primates, with special reference to the IIM and slow-tonic fibre types. J. Anatomy 137 (1), 95–108.
- Mason, M.J., 2015. Functional morphology of rodent middle ears. In: Evolution of the Rodents: Advances in Phylogeny, Functional Morphology and Development. Cambridge University Press, pp. 373–404. doi:10.1017/CB09781107360150.015.
- Mason, M.J., 2016. Structure and function of the mammalian middle ear. I: large middle ears in small desert mammals. J. Anatomy 228 (2), 284–299. doi:10.1111/ joa.12313.
- Milazzo, M., Fallah, E., Carapezza, M., Kumar, N.S., Lei, J.H., Olson, E.S., 2017. The path of a click stimulus from ear canal to umbo. Hear. Res. 346, 1–13. doi:10.1016/j. heares.2017.01.007.
- Miyawaki, H., Nakayama, A., Hiryu, S., Kobayasi, K.I., Riquimaroux, H., 2015. Auditory sensitivity shift by attention in Mongolian gerbil. J. Acoust. Soc. Am. 138 (3), 1792. doi:10.1121/1.4933684.

- Møller, A.R., 1964. Effect of tympanic muscle activity on movement of the eardrum, acoustic impedance and cochlear microphonics. Acta Oto-Laryngol. 58, 1–10. doi:10.3109/00016486409121413.
- Møller, A.R., 1965. An experimental study of the acoustic impedance of the middle ear and its transmission properties. Acta Oto-Laryngol. 60, 129–149. doi:10. 3109/00016486509126996.
- Møller, A.R., 1984. Neurophysiological Basis of the Acoustic Middle-Ear Reflex. In. The Acoust. Reflex 1–34. doi:10.1016/b978-0-12-643450-7.50006-8.
- Møller, A.R., Moore, B.C.J, 2001. Hearing: Its Physiology and Pathophysiology. Academic Press, Inc.
- Mukerji, S., Windsor, A.M., Lee, D.J., 2010. Auditory brainstem circuits that mediate the middle ear muscle reflex. Trends Amplif. 14 (3), 170–191. doi:10.1177/ 1084713810381771.
- Murphy, D.L., King, C., Schlebusch, S., Shera, C., Groh, J., 2020. Evidence for a system in the auditory periphery that may contribute to linking sounds and images in space. BioRxiv (Preprint) 1–31. doi:10.1101/2020.07.19.210864.
- Nakayama, A., Riquimaroux, H., 2017. Sensitivity to high frequency communication sounds in the inner ear enhanced by selective attention: preliminary findings in mongolian gerbils. J. Comput. Acoust. 25 (3), 1–11. doi:10.1142/ S0218396X17500163.
- Nishiyama, K., Kobayasi, K.I., Riquimaroux, H., 2011. Vocalization control in Mongolian gerbils (Meriones unguiculatus) during locomotion behavior. J. Acoust. Soc. Am. 130 (6), 4148–4157. doi:10.1121/1.3651815.
- Nuttall, A.L., 1974. Tympanic muscle effects on middle-ear transfer characteristic. J. Acoust. Soc. Am. 56 (4), 1239–1247. doi:10.1121/1.1903414.
- Overstreet, E.H., Ruggero, M.A., 2002. Development of wide-band middle ear transmission in the Mongolian gerbil. J. Acoust. Soc. Am. 111 (1), 261–270. doi:10. 1121/1.1420382.
- Politzer, A., 1909. A textbook of the diseases of the ear. english translation by. In: Ballin, Milton J., Heller, Clarence L. (Eds.), The Boston Medical and Surgical Journal, Fifth edition In. The Boston Medical and Surgical Journal doi:10.1056/ nejm190912301612709.
- Puria, S., Allen, J.B., 1998. Measurements and model of the cat middle ear: evidence of tympanic membrane acoustic delay. J. Acoust. Soc. Am. 104 (6), 3463–3481. doi:10.1121/1.423930.
- Ramírez, L.M., Ballesteros, L.E., Sandoval, G.P., 2007. Tensor tympani muscle: strange chewing muscle. Medicina Oral, Patología Oral y Cirugía Bucal 12 (2), 96–100.
- Ravicz, M.E., Cooper, N.P., Rosowski, J.J., 2008. Gerbil middle-ear sound transmission from 100Hzto60kHz. J. Acoust. Soc. Am. 124 (1), 363–380. doi:10.1121/1. 2932061.
- Ravicz, M.E., Rosowski, J.J., Voigt, H.F., 1992. Sound-power collection by the auditory periphery of the Mongolian gerbil Meriones unguiculatus . I: Middle-ear input impedance. J. Acoust. Soc. Am. 92 (1), 157–177. doi:10.1121/1.404280.
- Rock, E., 1995. Objective tinnitus and the tensor tympani muscle. Int. Tinnitus J. 1 (1), 30–37.
- Rosowski, J.J., 2003. The middle and external ears of terrestrial vertebrates as mechanical and acoustic transducers. In: Sensors and Sensing in Biology and Engineering. Springer, pp. 59–69. doi:10.1007/978-3-7091-6025-1_5.
- Rosowski, J.J., Ravicz, M.E., Teoh, S.W., Flandermeyer, D., 1999. Measurements of middle-ear function in the Mongolian gerbil, a specialized mammalian ear. Audiol. Neuro-Otol. 4 (3-4), 129–136. doi:10.1159/000013831.
- Ryan, A., 1976. Hearing sensitivity of the mongolian gerbil, Meriones unguiculatis. J. Acoust. Soc. Am. 59 (5), 1222–1226. doi:10.1121/1.380961.
- Smith, H., 1943. Audiometric effects of voluntary contraction of the tensor tympani muscles. Arch Otolaryngol 19 (369), 72.
- Teoh, S.W., Flandermeyer, D.T., Rosowski, J.J., 1997. Effects of pars flaccida on sound conduction in ears of Mongolian gerbil: Acoustic and anatomical measurements. Hear. Res. 106 (1–2), 39–65. doi:10.1016/S0378-5955(97)00002-6.
- Van der Heijden, M., Joris, P.X., 2003. Cochlear phase and amplitude retrieved from the auditory nerve at arbitrary frequencies. J. Neurosci. 23 (27), 9194–9198. doi:10.1523/jneurosci.23-27-09194.2003.
- Veggetti, A., Mascarello, F., Carpenè, E., 1982. A comparative histochemical study of fibre types in middle ear muscles. J. Anatomy 135 (Pt 2), 333–352.
- Voss, S.E., Rosowski, J.J., Merchant, S.N., Peake, W.T., 2001. How do tympanicmembrane perforations affect human middle-ear sound transmission? Acta Oto-Laryngol. 121, 169–173. doi:10.1080/000164801300043343.
- Wickens, B., Floyd, D., Bance, M., 2017. Audiometric findings with voluntary tensor tympani contraction. J. Otolaryngol. Head Neck Surg. 46 (1), 1–5. doi:10.1186/ s40463-016-0182-y.
- Wolf, M., Schuchmann, M., Wiegrebe, L., 2010. Localization dominance and the effect of frequency in the Mongolian Gerbil, Meriones unguiculatus. J. Compar. Physiol. A: Neuroethol. Sens. Neural Behav. Physiol. 196, 463–470. doi:10.1007/ s00359-010-0531-7.
- Zheng, Y., Ohyama, K., Hozawa, K., Wada, H., Takasaka, T., 1997. Effect of anesthetic agents and middle ear pressure application on distortion product otoacoustic emissions in the gerbil. Hear. Res. 112 (1–2), 167–174. doi:10.1016/ S0378-5955(97)00118-4.