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Investigation to Examine the Profile of Auditory Brainstem and Hearing Thresholds Using Tone Burst Audiometry Brainstem Response in a Preclinical Migraine Model

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INVESTIGATION TO EXAMINE THE PROFILE OF AUDITORY BRAINSTEM AND HEARING THRESHOLDS USING TONE BURST AUDIOMETRY BRAINSTEM RESPONSE IN A PRECLINICAL MIGRAINE MODEL

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Doctor of Audiology, Communication Sciences and Disorders

By

Megan Huelsing

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ABSTRACT

Migraine is a disabling neurological disease that is characterized by prominent auditory symptoms, including hyperacusis, which is defined as extreme sensitivity to sounds. The goal of this study was to investigate changes in hearing sensitivity and neural changes in the brainstem by measuring neural activity of the auditory brainstem in an established preclinical model of chronic migraine. To induce a chronic migraine state within the trigeminal system, male and female Sprague Dawley rats were subjected to three known human risk factors including neck muscle tension and REM sleep deprivation that promote latent sensitization, and exposure to a pungent odor, which acts as a trigger to stimulate trigeminal activation and pain signaling. Tone burst auditory brainstem responses (ABRs) were measured using the Duet device (Intelligent Hearing Systems, Miami, FL) to determine the effects of migraine pathology on brainstem auditory pathways in eight male and six female rats at baseline (naïve) condition and post migraine condition. Tone burst ABR was recorded in each ear to 4 kHz, 12 kHz, 22 kHz, and 32 kHz stimuli. To assess neural changes in the brainstem, suprathreshold ABRs were recorded at 80 dB sound pressure level and waveform morphology, latency, and amplitude responses were analyzed. To determine the threshold, tone burst ABR was recorded at 50 dB and 20 dB and then at 30 dB or 10 dB depending on the response at 20 dB. The threshold value was defined as the lowest intensity to elicit a reliable wave II, which is the largest wave in rats. At baseline (naïve condition), the 80 dB ABR morphology exhibited the presence of distinct waves I, II, and III at lower frequencies (4 kHz and 12 kHz) and waves I, II, III, IV, and V at higher frequencies (22 kHz and 32 kHz), with wave II being the largest wave. As the frequencies increased from 4 kHz to 32 kHz, latencies decreased, and amplitudes increased with a larger amplitude observed in the right ear at 4 kHz and 12 kHz (wave II mean = 1.98 μV) compared to the left ear (wave II mean = 1.65 μV). After induction of migraine pathology, ABR thresholds were elevated to mild-moderate degree, latencies decreased at 4 kHz, 12 kHz, 22 kHz, and 32 kHz and amplitudes increased, mainly wave II, at 4 kHz and 32 kHz. Results from this novel study provide, to our knowledge, the first evidence of neural changes in auditory brainstem response and changes in high frequency hearing sensitivity in a preclinical chronic migraine model.

KEYWORDS: migraine, hearing sensitivity, hyperacusis, rat, auditory brainstem response

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INTRODUCTION

Migraine

Migraine is a disabling neurological disease with different associated symptoms when compared to just a headache [1, 2]. Migraine affects 12% of the population and is the second most disabling condition worldwide and may affect all aspects of a person's life, including occupational, academic, social, and family [3-5]. It affects women disproportionally more than men at a ratio of 3:1 [6], presumably the result of sex hormones that are known to alter brain function and neuronal excitability [7, 8]. Migraine can be classified as episodic or chronic; episodic migraine occurs 14 days or less per month and chronic migraine occurs 15 or more days per month for three or more months [2, 9]. Common symptoms of migraine are moderate to severe head pain, nausea, sensitivity to light (photophobia), noise (phonophobia), or smells, and is severe enough to make you miss school or work.

Migraine Auditory Symptoms

Many auditory symptoms are associated with migraine [10]. Noise sensitivity, fluctuating low frequency hearing loss, sudden hearing loss, persistent tinnitus, aural fullness, otalgia, dizziness, and sinus symptoms have all been reported during migraine attacks [11]. The most prominent of these auditory symptoms during a migraine is noise sensitivity. About two thirds of patients who suffer from migraines report noise sensitivity and state that a loud noise can trigger a migraine attack [12]. Schwedt [13] states that increasing severity of pain is associated with greater sensitivity to sound, and migraineurs have a lower sound discomfort threshold. This

extreme sensitivity to sound during a migraine attack may be due to lower hearing thresholds and higher brainstem neuronal excitability [8].

Phonophobia and hyperacusis are two separate, yet closely related auditory symptoms that are often mistakenly used in clinical practice in the same entity to refer to hypersensitivity to sound [14]. Phonophobia is defined as a persistent, abnormal, and unwarranted fear of sound, and is an anxiety disorder and not a hearing disorder [14]. Hyperacusis is defined as a disorder in loudness perception, and patients may appear overly sensitive to a range of sounds, finding noises unbearable or painful [15]. The migraine field uses the term phonophobia to describe sensitivity to sound during a migraine attack, however the more correct term would be loudness hyperacusis or pain hyperacusis as a subtype of hyperacusis [16]. This highlights a knowledge gap and lack of understanding of this auditory manifestation in the field of headaches and migraine. As defined in the paper by Suhnan et al., [17] hyperacusis can be a debilitating experience but is an under-recognized symptom and an integral feature in chronic migraine. Hypersensitivity to sound is most prominent during individual migraine attacks, however some migraineurs have less prominent but persistent hypersensitive to stimuli between migraine attacks [13, 18]. When migraineurs have exposure to a specific sensory stimulus they experience hypersensitivity not only to that stimulus but may also have further enhancement of hypersensitivities to other sensory stimuli (e.g., light) that may contribute to a worsening headache intensity. Understanding the interactions that occur when processing multiple external stimuli and the activation of the trigeminal system may help explain migraine symptoms and mechanics [18]. Nociceptive circuits become hypersensitive in chronic pain, and this sensitivity spreads from the periphery to spinal neurons and higher centers in the brain, leading to

spontaneous pain. This central sensitization may alter activity at sensory convergence points in the thalamus and brainstem centers and give rise to hyperacusis in central pain syndromes [17]. Along with noise sensitivity, migraineurs may also experience low-frequency fluctuating hearing loss or sudden hearing loss. Baloh [11] reported patients with migraine had an abrupt onset of profound sensorineural hearing loss and some showed gradual improvement. However, most patients are often left with severe unilateral sensorineural hearing loss. Viirre and Baloh [19] reported eleven of thirteen patients with migraine had an acute onset of unilateral sensorineural hearing loss. These sudden episodes of sensorineural hearing loss associated with migraine could be explained by vasospasms of the cochlear branch of the internal auditory artery or because of a defective calcium channel shared by the brain and the inner ear that leads to reversible hair cell depolarization [11].

Therefore, more research is needed to understand how migraine affects the inner ear and nerve conduction and processing since migraine is considered a central nervous system disorder [9, 10, 20]. The objective of this proof-of-concept study was to determine if auditory changes at the brainstem level could be detected in an established preclinical model of chronic migraine potentially providing an animal model migraine-induced hearing loss and hyperacusis. Such a model could be used to develop therapeutic strategies to inhibit the auditory disorders in migraineurs.

Auditory Brainstem Response

The auditory brainstem response (ABR) is a potentially useful tool to study changes in auditory brainstem function in individuals experiencing chronic migraine. The ABR is an objective, non-invasive technique, far-field recording that has been used for decades to

assess hearing thresholds and the peripheral and central auditory pathway up to the level of the inferior colliculus. Findings from human studies have provided evidence that an abnormal ABR may be the earliest auditory indication in migraine since migraineurs with auditory symptoms tend to have prolonged peak latencies and/or interpeak latencies [21]. Hamed et al., [22] found that nearly two-thirds of migraine patients had abnormalities in ABR electrophysiological testing, including prolonged wave III latencies and I-V interpeak latencies. These results indicate that subclinical changes in cochlear function and the auditory brainstem are associated with chronic migraine [22].

Rats are one of the most widely used experimental models in hearing; rats and humans share similar cochlear physiology and anatomy, such as having two and a half cochlear turns [23, 24]. The rat ABR is comprised of five waves (I, II, III, IV, and V) that reflect the synchronous short-latency synaptic activity along the brainstem auditory pathway and appear within six milliseconds after the onset of an auditory stimulus. The ABR generators in rats that reflects the serial transmission neuronal activity along the brainstem from the auditory nerve, cochlear nucleus, superior olivary complex, lateral lemniscus, and inferior colliculus, believe to correspond to waves I, II, III, IV, and V [25, 26]**.**

Research has been conducted to study the characteristics of the pattern of ABR waves in male Wistar rats to establish baseline criteria that could be used to identify abnormalities [25]. Threshold tone burst ABR in rats at seven different frequencies from 500 to 32 kHz and ABR waves were classified based on morphology. It was found that the morphology of the rat ABR and human ABR have some noticeable differences, which can be seen in Figure 1. In rats, wave II of the ABR is the most prominent, while in humans, wave V is the largest. For this reason, wave II in rats and wave V in human are typically used to determine ABR hearing

thresholds. Rat ABR wave latencies decreased, and amplitudes increased as the stimulus level increased; however, there were frequency-dependent variations in the patterns of ABR waveforms [25]. When stimulus frequency decreased from 8000 to 2000 Hz, ABR latencies increased [25, 26]. These changes reflect a shift in the peak of the traveling wave towards the apex (increased travel time). ABR latency also increased as intensity decreased due to a reduced spread of excitation along the basilar membrane, less number of neural elements firing, and a reduction in neural synchrony [26]. Overbeck et al., [26] found that rats have larger ABR amplitudes than humans at 8000 Hz whereas humans have larger responses at 2000 Hz; these differences presumably reflect the fact that the range of hearing in rats is roughly 1-octave higher in rats than humans (need ref). Although rats have better hearing acuity at ultra-high frequencies above 8000 Hz, Overbeck did not assess ABR responses at frequencies higher than 8000 Hz. The fact that Overbeck et al., [26] did not assess frequencies higher than 8000 Hz represents a gap in the field of chronic migraine and ABR.

Goal of the Study

Although there has been much research in humans discussing the effects of episodic and chronic migraine on hearing sensitivity, the results have been inconclusive [8]. There are also no studies to our knowledge that have evaluated hearing thresholds in a preclinical chronic migraine model. In order to study the frequency- and intensity-dependent changes in ABR function during chronic migraine, it was necessary to measure ABR thresholds, amplitudes and neural transmission times over a broad range of frequencies and intensities. Therefore, ABR thresholds, and ABR morphology, peak latencies, and amplitudes were measured before and after induction of chronic migraine in young male and female rats. Because migraine is more common in

females than males, measurements were obtained from both genders to test for migraine-induced differences in ABR activity in males versus females.

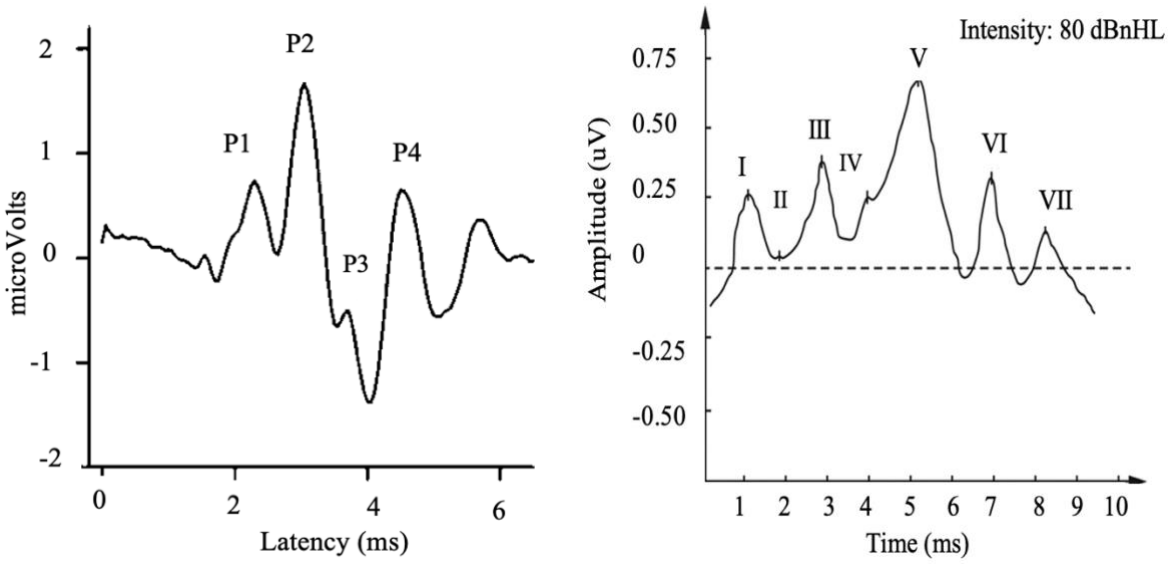

Figure 1. Representative tracings of a rat click ABR (left) and human click ABR (right).

METHODS

Animals

Sprague Dawley male (eight) and female (six) rats (200-300 g) were housed at a temperature of 72°F, with 12-hour light/dark cycles, humidity between 30-70%, and air changes between 10-15 per hour. The animals were monitored daily by staff and weekly by an attending veterinarian. Cage changes were performed on average once per week. Animals were allowed unrestricted access to food and water. All procedures were approved by Missouri State University's IACUC committee, see appendix (2021-18 IACUC approved 9/14/2021). Prior to entry into this study, the hair located on the submandibular area was removed using Nair™. Animals were lightly anesthetized under 3% isoflurane mixed in oxygen, and the hair was trimmed using standard beard trimmers. Once the bulk of the hair was removed, the area was wetted and Nair™ was placed upon the area of interest using a cotton swab. Removal was allowed to occur for 4 minutes, at which point the area was generously washed to ensure all the Nair™ product was removed from the animal. Animals were allowed to recover in their normal cages.

Migraine Procedure

Inducing Trigeminal Sensitization. Establishment of the chronic migraine model was done using the methodology described in detail in a prior published study [27] and outlined in Figure 2. Animals were placed under 3% isoflurane with oxygen as the carrier gas and injected with complete Freund's adjuvant (CFA; 1:1 in saline; 10 µl each, Sigma-Aldrich, St. Louis, MO) in 10 separate locations in the trapezius muscle to promote prolonged inflammation and neck

muscle tension/tenderness. Animals were allowed to recover in their cages and monitored for normal grooming behaviors prior to being sleep deprived.

REM Sleep Deprivation. Rapid eye movement (REM) sleep deprivation was induced by placing animals on a 3-inch x 3-inch x 3-inch platform that was surrounded by room temperature water. The purpose of such a platform was to prevent the animal from fully entering a REM sleep cycle. Once an animal reached REM sleep, they lost muscle control which caused a loss of balance and ultimately resulted in arousal when the animals came in contact with the water. The animals were sleep deprived for 24 hours and placed in fresh-bedded cages for 30 minutes prior to nocifensive assessment.

Triggering Trigeminal Nociceptor Activation Via Exposure to Pungent Odor. To prepare the oil extract, raw California Bay leaves (CBL) were dried and crushed. The bulk, volatile oil extract was obtained through standard steam isolation (See AOAC Official Method 962.17). Seven days after sleep deprivation, animals were placed in a 3.3 L box with oxygen flow at 2 L/min. A cotton swab with 20 µl of extracted leaf oil was placed in an isolated tube within the box so that the rats could not make direct contact with the stimulant but were exposed to the volatile compounds concentrated in the extracted oil. Animals were allowed to breathe the air for a total of 10 minutes that was permeated with volatile organic compounds, including umbellulone, which is known to cause trigeminal activation and migraine-like pain [28, 29]. After exposure to the bay leaf extract, animals were returned to their normal cages.

Trigeminal Nociception in Response to Mechanical Stimulation. Animals were placed in a Durham animal holder (UGO Basile) for 5 minutes for 3 consecutive days prior to all behavioral studies. A range of von Frey filaments were applied to the cutaneous area over the temporalis (V1 region) or masseter (V3 region) areas 5 times (Figure 3). Head withdrawal

responses to mechanical stimulation were recorded out of 5 applications on each side (describe your rating scale, for example, no response =1, strong response 6). Data were reported using the average number of nocifensive responses of both sides. Baseline responses were taken 24 hours before testing experimental conditions. Nocifensive measurements were taken at 2 post exposure to CBL extract. In some instances, sensitized animals exposed to CBL exhibited an unresponsive behavior to even the 180 g filament in which case those animals were given a value of 6. Statistical significance was determined by a Mann-Whitney U t-Test using SPSS software (release 21.0). Differences were considered significant when $p < 0.05$.

Auditory Brainstem Response (ABR) Threshold Protocol

Recording and Stimulus Parameters. Tone bursts ABRs were measured to determine effects of migraine on auditory brainstem neural firing and ABR thresholds. The 2-channel ABR was recorded between four needle electrodes inserted subcutaneously using the Duet device (Intelligent Hearing Systems, Miami, FL). The following recording and stimulus parameters are summarized in Table 1a and Table 1b. The active electrode was placed at the vertex (Cz), the reference electrodes behind the left ear (A1- channel 1) and the right ear (A2- channel 2), and the ground electrode at the base of the tail (see Figure 4). The ABRs were amplified by a factor of 100 K and a digital bandpass filter of 30-3000 Hz was used. The amplified signals were averaged with at least 256 sweeps at 80 dB SPL and up to 3672 sweeps at 10 dB SPL. The stimulus repetition rate was 21.1/s to have robust resolution of ABR morphology [24]. An analysis epoch of 12 ms was used.

ABR Recording Procedure. ABRs were collected from anesthetized rats. All electrode and inter-electrode impedance were low (< 5 kOhms) and balanced. ABRs were recorded on four

sessions: at baseline and at day 1, 14, and 22 post chronic migraine induction. Stimuli of 4, 12, 22, and 32k H tone bursts were generated through Animal High Frequency Transducers (Intelligent Hearing Systems, Miami, FL) with insert tubes placed into the left and right ear canal. ABR recordings were obtained monaurally from the right ear and the left ear of all rats. Serial ABRs were collected to a series of stimulus intensities starting at 80 dB SPL and then decreasing to 50, 20, and then to 30- or 10-dB SPL depending on the response at 30 dB SPL. Two to three traces of the ABR were collected and then averaged and labeled. ABR Threshold was defined as the lowest stimulus intensity to elicit a reliable wave II. Two raters, one of them is an expert in auditory electrophysiology including ABR, labelled ABR averaged traces, and the interrater reliability was 95%.

Table 1a. ABR Recording Parameters

2- channel	Cz-A1/A2; tail (active-reference; ground)
Amplification Gain	100 K
Bandpass filter	30-3000 Hz
Repetion Rate	21.1/sec
Analysis Window	12 ms

Table 1b. ABR Stimulus Parameters

Type	4, 12, 22, and 32 kHz Toneburst
Duration	100 microseconds
Polarity	Alternating
Intenstity	80 dB SPL, decreasing to 50, 20, and then to 30- or 10-dB SPL
Sweeps	256 at 80 dB, 512 at 50 dB, 1024 at 30 dB, and 3672 at 20 dB and 10 dB

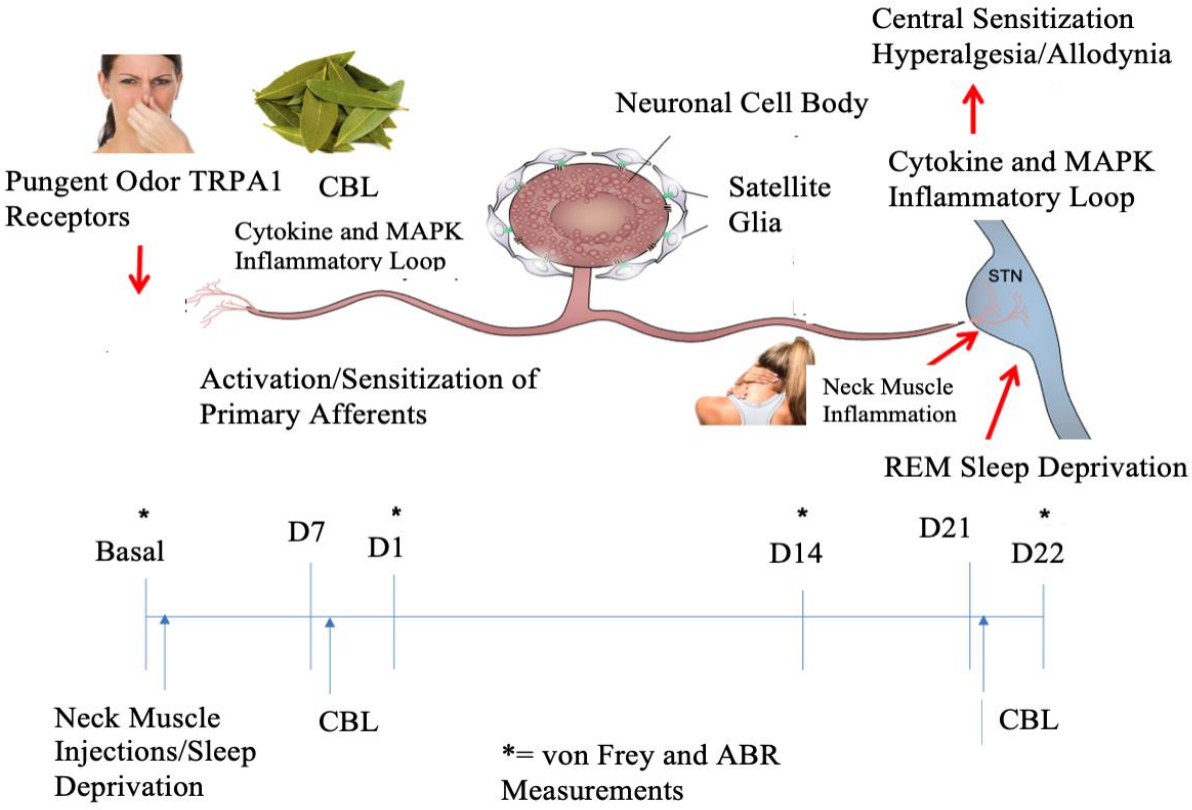

Figure 2. Schematic model of induction of chronic migraine phenotype in Sprague Dawley rats and experimental timeline. Neck muscle injections and sleep deprivation were performed immediately following basal testing. On day 7 after sleep deprivation, California Bay Leaf (CBL) exposure was performed. CBL exposure was performed a second time 21 days later. Asterisks indicate von Frey and hearing testing times, which were performed basally, and then on days 1, 14, and 22 after CBL exposure. See text for a more detailed description of the methodology.

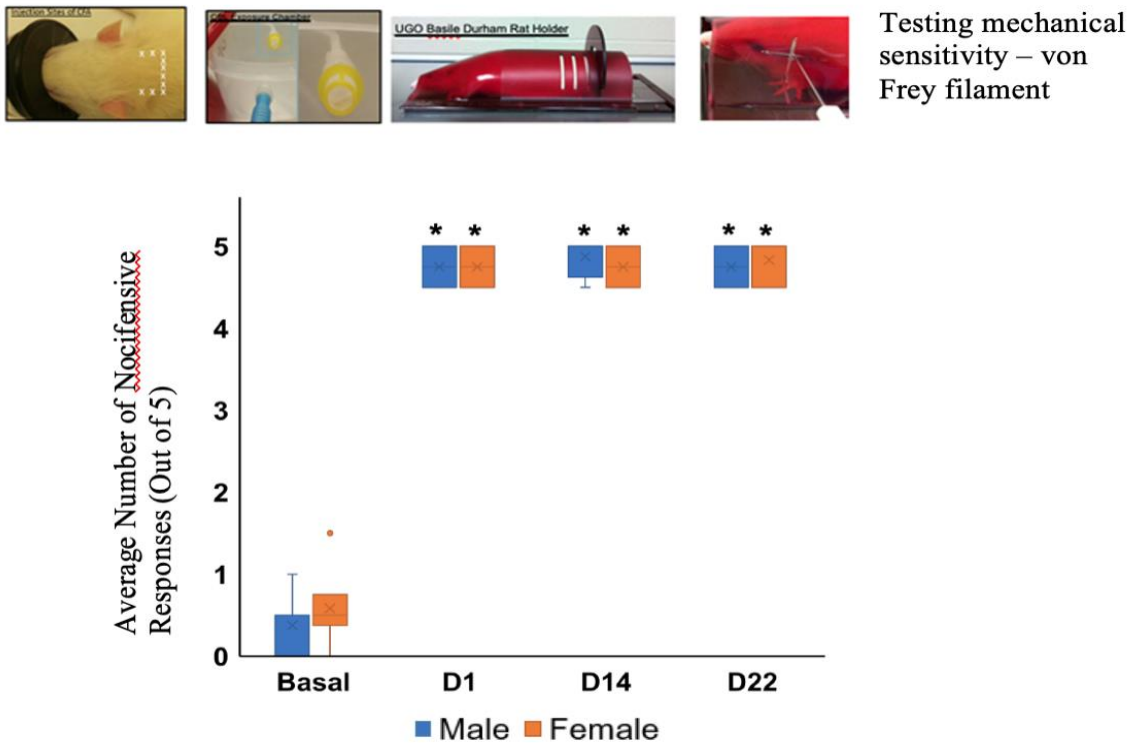

Figure 3. Overview of techniques used to establish migraine-like phenotype and measurement of nocifensive responses. (Top) The sites of CFA injection into the trapezius muscle that induce sensitization are shown in the far-left panel. Next is shown how the pungent odor was delivered to the rats via continuous airflow. The two right panels show the placement of the rat in the Durham holder and the location of testing for mechanical sensitivity using von Frey filaments. (Bottom) The chronic migraine model results in increases in nocifensive responses. Neck muscle injections and sleep deprivation were performed immediately following basal testing. On day 7 after sleep deprivation, California Bay Leaf (CBL) exposure was performed. CBL exposure was performed a second time 21 days later. * $P < 0.05$ compared to basal levels.

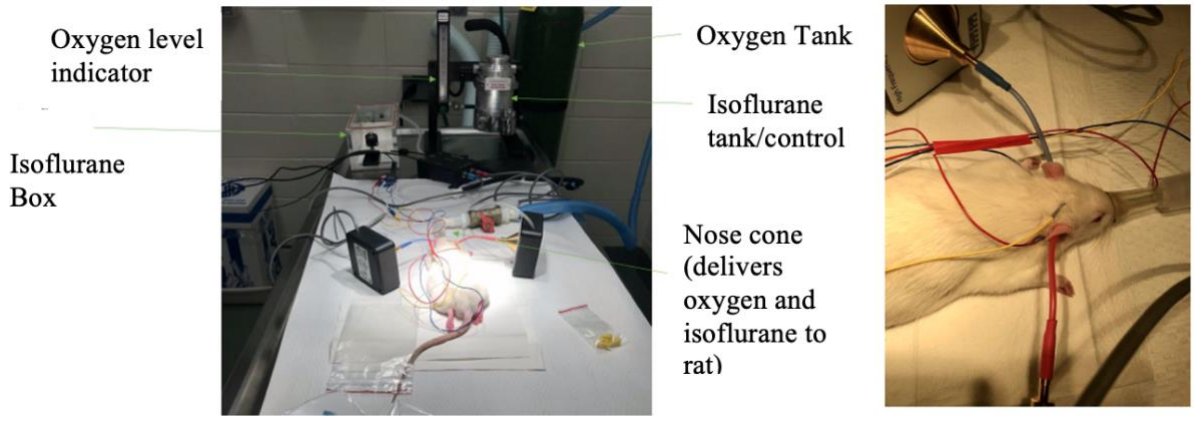

Figure 4. Overview of experimental setup of ABR testing. See text for full details.

RESULTS

Male ABR Morphology

Baseline Pre-Migraine. Baseline ABRs were first recorded in the rats before migraine induction to assess normative characteristics of moprhology, latency, and amplitude. Baseline ABR averages from all eight male rats obtained at 80 dB SPL at 4 kHz, 12 kHz, 22 kHz, and 32 kHz for the left and right ear are shown in Figure 5. The ABR measurements were similar in morphology to those recorded by other researchers. Both the left and right ears have similar ABR morphology, latency, and amplitude at each frequency tested. Overall, when comparing recorded ABR waveforms between frequencies different wave morphologies were noticed. As shown in Figure 5, waves I, II, and III are present at all four tested frequencies in both the left and right ear. Then, wave IV appears at 12 kHz, 22 kHz, and 32 kHz, and wave V appears at 22 and 32 kHz, the most sensitive frequencies in rats.. At 4 kHz and 12 kHz, each wave is it's own separate wave. However, at 22 kHz, waves I, II, and III form a characteristic W-shape pattern, and wave IV is a notch on wave III followed by a small wave V. At 32 kHz, all five waves are recorded but the peak of wave III is close to wave II. Finally, a small wave V is present in both the left and right ear at 12 kHz, 22 kHz, and 32 kHz, with a relatively larger amplitude at 32 kHz; wave V is not recorded at 4 kHz. Given that wave II is the largest wave in both ears at all frequencies, it was used to determine changes in ABR threshold.

4 kHz Post Migraine. After baseline ABR normative characteristics were recorded, ABRs were then measured in the rats at day 1, day 14, and day 22 post migraine induction to compare moprhology, latency, and amplitude to asses if the migraine treated affects any of these baseline characterisitcs. Figure 6 shows baseline, day 1, day 14, and day 22 ABR morphology

for 4 kHz. At baseline, waves I, II, and III are present. At day 1, day 14 and day 22 post-migraine, waves I, II, and III are readily observed with enhanced amplitudes and earlier absolute latency, and wave IV becomes visible post-treatment.

12 kHz Post Migraine. Figure 7 shows baseline, day 1, day 14, and day 22 ABR morphology for 12 kHz. At baseline, waves I, II, and III are present. At day 1, day 14, and day 22 post-migraine treatment, waves I, II, III, and IV are readily observed with enhancement of the response amplitude and earlier absolute latencies of the first three waves compared to baseline.

22 kHz Post Migraine. Figure 8 shows baseline, day 1, day 14, and day 22 ABR morphologyfor 22 kHz of both ears. At baseline, waves I, II, III, IV, and V are present. The ABR waveform stayed consistent through the post-migraine period and showed no morhplogy changes. At day 1, day 14, and day 22 post-migraine, ABRs showed larger response amplitudes of all waves, except wave V, mainly on the right ear compared to the left ear. Also, earlier latencies were noticed on day 14 and day 22 post migraine.

32 kHz Post Migraine. Figure 9 shows baseline, day 1, day 14, and day 22 ABR morphology for 32 kHz. At baseline, waves I, II, III, IV, and V are all present. There were no evident morphology changes for 32 kHz for either the left or right ear during the post-migraine period. Earlier latency of 32 kHz waves were noticed on day 14 and day 22 post migraine.

Male ABR Latency

Baseline Pre-Migraine. Because chronic migraine treatments altered the latenices of the ABR peaks, the absolute peak latencies (PLs) at each freqeuncy, migraine condition, and ear are compared in detail to identify changes in speed of transmission of the neurons from the auditory

nerve up to the inferior colliculus. The average ABR latencies at 80 dB for waves I, II, III, IV, and V are shown in Table 2a (right ear) and Table 2b (left ear).

4 kHz Post Migraine. The mean latencies for all eight males for waves I-V at each condition at 4 kHz tested are shown in Table 3a (right ear) and Table 3b (left ear). For wave I, the largest latency change was seen in the right ear at day 14 when the latency decreased by 0.13 ms. For wave II, the largest latency change was seen in the right ear at day 14 when the latency decreased by 0.15 ms. For wave III, the largest latency change was seen in the right ear at day 14 when the latency decreased by 0.28 ms. For wave IV, the largest latency change was seen in the right ear at day 14 when the latency increased by 0.08 ms. Wave V was not present in either ear at any condition tested. Figure 10 shows an average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 4 kHz.

12 kHz Post Migraine. The mean latencies for all eight males for waves I-V at each condition at 12 kHz tested are shown in Table 4a (right ear) and Table 4b (left ear). For wave I, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.08 ms. For wave II, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.17 ms. For wave III, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.81 ms. For wave IV, the largest latency change was seen in the right ear at day 22 when the latency decreased by 1.56 ms. For wave V, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.42 ms. Figure 10 shows an average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 12 kHz.

22 kHz Post Migraine. The mean latencies for all eight males for waves I-V at each condition at 22 kHz tested are shown in Table 5a (right ear) and Table 5b (left ear). For wave I,

the largest latency change was seen in the left ear at day 1 when the latency increased by 0.14 ms. For wave II, the largest latency change was seen in the left ear at day 1 when the latency increased by 0.13 ms. For wave III, the largest latency change was seen in the right ear at day 22 when the latency decreased by 0.25 ms. For wave IV, the largest latency change was seen in the right ear at day 14 when the latency increased by 0.16 ms. For wave V, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.19 ms. Figure 10 shows an average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 22 kHz.

32 kHz Post Migraine. The mean latencies for all eight males for waves I-V at each condition at 32 kHz tested are shown in Table 6a (right ear) and Table 6b (left ear). For wave I, the largest latency change was seen in the right ear at day 14 when the latency increased by 0.28 ms. For wave II, the largest latency change was seen in the left ear at day 1 when the latency increased by 0.14 ms. For wave III, the largest latency change was seen in the left ear at day 1 when the latency increased by 0.29 ms. For wave IV, the largest latency change was seen in the left ear at day 1 when the latency increased by 0.27 ms. For wave V, the largest latency change was seen in the right ear at day 1 when the latency decreased by 0.37 ms. Figure 10 shows an average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 32 kHz.

Male ABR Amplitude

Baseline Pre-Migraine. The amplitude of individual ABR peaks is primarily a reflection of the number of neurons firing and/or degree of neural synchrony. To measure amplitude, the peak and trough of each wave was marked. The amplitudes of each ABR peak were measured

pre- and post- migraine treatment at each frequency. Wave II had the largest amplitude in each ear for all freqeuncies and all conditions.

4 kHz Post Migraine. Figure 11 (right ear) and figure 12 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 4 kHz. At 4 kHz, wave I amplitude increased from baseline to day 14, bilaterally. Wave II amplitudes increased steadily from baseline to day 22, bilaterally. Wave III, IV, and V showed no significant amplitude changes.

12 kHz Post Migraine. Figure 11 (right ear) and figure 12 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 12 kHz. Wave I amplitudes increased from baseline to day 14, bilaterally. Wave II amplitdes decreased from baseline to day 22, bilaterally. Wave III had a large amplitude shift at day 14 and 22, bilaterally. Waves IV and V had no significant amplitude changes.

22 kHz Post Migraine. Figure 11 (right ear) and figure 12 (left ear) show baseline, day 1, day14, and day 22 ABR amplitude changes for 22 kHz. Wave I did not show significant amplitude changes. The left ear wave II amplitudes decreased at day 1, but then increased at day 14. The right ear wave amplitudes increased from baseline to day 22. Wave IV amplitudes increased from baseline to day 22, bilaterally. Wave V in the right ear did not show any significant amplitude changes, but the left ear had a large amplitude change from baseline to day 1, which may be considered an outlier.

32 kHz Post Migraine. Figure 11 (right ear) and figure 12 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 32 kHz. Wave I and V amplitudes decreased from baseline to day 22, bilaterally. The left ear wave II amplitudes increased from baseline to day 22 and the right ear wave II amplitudes increased at day 1 and day 22 but decreased at day 14. Wave III and IV amplitudes increased from baseline to day 22, bilaterally.

Male ABR Threshold

Examples of the shift in threshold from baseline, day 1, day 14, and day 22 post migraine at 4, 12, 22, and 32 kHz for the right and left ear are shown in Figure 13. At the baseline measurement, as freqeuncies increased from 4 kHz to 32 kHz, thresholds decreased, indicating that the male rats have better hearing sensitivity at higher frequencies. After the chronic migraine was introduced, thresholds increased at each frequency per condition, bilaterally. This indicates that during a chronic migraine state, the rats had decreased hearing sensitivity and mild-moderate hearing loss. The largest threshold shift in the left ear was seen at 22 kHz from baseline to day 22 with a 25 dB increase in hearing threshold. The largest threshold shift in the right ear was seen at 12 kHz from baseline to day 22 with a 30 dB increase in hearing threshold. At all other frequencies, hearing thresholds increased an average of 10 dB from baseline to day 22, bilaterally.

Female ABR Morphology

Baseline Pre-Migraine. Baseline ABRs were first recorded in the rats before migraine induction to asses morphology, latency, and amplitude. Baseline ABR averages from all six female rats at 4 kHz, 12 kHz, 22 kHz, and 32 kHz for the left and right ear are show in Figure 14. The ABR measurements were similar in morphology to those recorded by other researchers. When comparing the left and right ear, they have similar morphology, latency, and amplitude at each frequency tested. As shown in Figure 14, waves I, II, III, and IV are present at all freqeuncies tested, and wave V is present at 12 kHz, 22 kHz, and 32 kHz. When comparing each frequency, different wave mophrology (shapes) were noticed. Wave II is the largest wave in both ears at all frequencies, and therefore was used to determine changes in threshold. At 4 kHz, each

wave is it's own separate wave. At 12 kHz and 32 kHz, wave III is a notch on wave II. At 22 kHz, wave IV is a notch on wave III.

4 kHz Post Migraine. After baseline ABR normative characteristics were recorded, ABRs were then measured in the rats at day 1, day 14, and day 22 post migraine induction to compare moprhology, latency, and amplitude to asses if migraine affects any of these baseline characterisitcs. Figure 15 shows baseline, day 1, day 14, and day 22 ABR morphology changes for 4 kHz. At baseline, waves I, II, III, and IV are present. Post migraine showed enhanced amplitudes and earlier latencies, especially in the right ear.

12 kHz Post Migraine. Figure 16 shows baseline, day 1, day 14, and day 22 ABR morphology changes for 12 kHz. At baseline, waves I, II, III, IV, and V are present. Wave III is a notch on wave II. The left and right ear stayed consistent through the course of the migraine and showed no morhplogy changes.

22 kHz Post Migraine. Figure 17 shows baseline, day 1, day 14, and day 22 ABR morphology changes for 22 kHz. At baseline, waves I, II, III, IV, and V are present. There were no morphology changes for 22 kHz for either the left or right ear throughout the course of the migraine. The right ear showed evident enhancement of waves post migraine.

32 kHz Post Migraine. Figure 18 shows baseline, day 1, day 14, and day 22 ABR morphology changes for 32 kHz. At baseline, waves I, II, III, IV, and V are all preseent. There were no morphology changes for 32 kHz for either the left or right ear throughout the course of the migraine.

Female ABR Latency

Baseline Pre-Migraine. A chronic migraine state caused differing results on the latenices of each wave at each freqeuncy per ear. The peak latencies (PLs) at each freqeuncy, migraine condition, and ear are compared in detail in the following paragraphs. The average ABR latencies at 80 dB for waves I, II, III, IV, and V are shown in Table 7a (right ear) and Table 7b (left ear).

4 kHz Post Migraine. The mean latencies for all six females for waves I-V at each condition at 4 kHz tested are shown in Table 8a (right ear) and Table 8b (left ear). For wave I, the largest latency change was seen in the left ear at day 1 when the latency decreased by 0.06 ms. For wave II, the largest latency change was seen in the right ear at day 22 when the latency decreased by 0.16 ms. For wave III, the largest latency change was seen in the left ear at day 1 when the latency decreased by 0.23 ms. For wave IV, the largest latency change was seen in the left ear at day 1 when the latency decreased by 0.50 ms. Wave V was not present in either ear at any condition tested. Figure 19 shows an average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 4 kHz.

12 kHz Post Migraine. The mean latencies for all six females for waves I-V at each condition at 12 kHz tested are shown in Table 9a (right ear) and Table 9b (left ear). For wave I, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.07 ms. For wave II, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.11 ms. For wave III, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.39 ms. For wave IV, the largest latency change was seen in the left ear at day 22

when the latency decreased by 0.44 ms. For wave V, the largest latency change was seen in the right ear at day 14 when the latency decreased by 0.28 ms. Figure 19 shows an average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 12 kHz.

22 kHz Post Migraine. The mean latencies for all six females for waves I-V at each condition at 22 kHz tested are shown in Table 10a (right ear) and Table 10b (left ear). For wave I, the largest latency change was seen in the left ear at day 1 when the latency increased by 0.05 ms. For wave II, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.06 ms. For wave III, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.23 ms. For wave IV, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.96 ms. For wave V, the largest latency change was seen in the left ear at day 1 when the latency decreased by 0.30 ms. Figure 19 shows an average latency change of the left and right ear combine at baseline, day 1, day 14, and day 22 ABR latency changes for 22 kHz.

32 kHz Post Migraine. The mean latencies for all six females for waves I-V at each condition at 32 kHz tested are shown in Table 11a (right ear) and Table 11b (left ear). For wave I, the largest latency change was seen in the right ear at day 1 when the latency increased by 0.12 ms. For wave II, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.16 ms. For wave III, the largest latency change was seen in the left ear at day 14 when the latency decreased by 0.21 ms. For wave IV, the largest latency change was seen in the left ear at day 22 when the latency decreased by 0.25 ms. For wave V, the largest latency change was seen in the left ear at day 1 when the latency decreased by 0.23 ms. Figure 19 shows an

average latency change of the left and right ear combined at baseline, day 1, day 14, and day 22 ABR latency changes for 32 kHz.

Female ABR Amplitude

Baseline Pre-Migraine. A chronic migraine state caused differing results on the amplitudes of each wave at each freqeuncy per ear. Measuring amplitude in an ABR is important as the amplitude indicates the relative number of neurons firing and/or degree of neural synchrony. To measure amplitude, the peak and trough of each wave was marked. The amplitudes at each freqeuncy, migraine condition, and ear are compared in detail in the following paragraphs. Wave II had the largest amplitude in each ear for all freqeuncies and all conditions.

4 kHz Post Migraine. Figure 20 (right ear) and figure 21 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 4 kHz. At 4 kHz, wave I amplitude increased from baseline to day 14, bilaterally. Wave II amplitudes increased significantly from baseline to day 14, bilaterally. Wave III, IV, and V had no significant amplitude changes.

12 kHz Post Migraine. Figure 20 (right ear) and figure 21 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 12 kHz. At 12 kHz, wave I amplitudes increased from baseline to day 14. Wave II amplitudes decreased from baseline to day 22. Wave III had a large amplitude shift at day 14 and 22. Waves IV and V had no significant amplitude changes.

22 kHz Post Migraine. Figure 20 (right ear) and figure 21 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 22 kHz. At 22 kHz, wave I did not show significant amplitude changes. The left ear wave II amplitudes decreased at day 1, but then increased at day 14. The right ear wave II amplitudes increased from baseline to day 22. Wave

IV amplitudes increased from baseline to day 22. Wave V did not show any major amplitude changes bilaterally.

32 kHz Post Migraine. Figure 20 (right ear) and figure 21 (left ear) show baseline, day 1, day 14, and day 22 ABR amplitude changes for 32 kHz. At 32 kHz, wave I decreased from baseline to day 22, bilaterally. The left ear wave II amplitudes increased from baseline to day 22 and the right ear wave II amplitudes decreased from baseline to day 22. Wave III decreased from baseline to day 22, bilaterally. Wave V did not show any major amplitude changes.

Female ABR Threshold

Examples of the shift in threshold from baseline, day 1, day 14, and day 22 post migraine at 4, 12, 22, and 32 kHz for the right and left ear for female rats are show in Figure 22. At the baseline measurement, as freqeuncies increased from 4 kHz to 32 kHz, thresholds decreased, indicating that the female rats have better hearing sensitivty at higher frequencies. After the chronic migraine was introduced, thresholds increased at each frequncy per conditon, bilaterally. This indicates that during a chronic migraine state, the rats had decreased hearing sensitivty and a mild-moderate hearing loss. The largest threshold shift in the left ear was seen at 22 kHz from baseline to day 22 with an 8 dB increase in hearing threshold. The largest threshold shift in the right ear was seen at 22 kHz from baseline to day 1 with a 9 dB increase in hearing threshold. At all other frequencies, hearing thresholds increased an average of 5 dB from baseline to day 22, bilaterally.

Table 2a. Mean Baseline Tone Burst ABR Latency (ms) for Right Ears in Male Rats

Frequency	I	II	III	IV	V
4 kHz	2.21	3.07	4.73	5.84	6.91
12 kHz	2.04	2.93	4.11	5.25	5.97
22 kHz	1.77	2.65	3.56	4.35	5.62
32 kHz	2.11	3.05	3.75	5.00	6.09

Table 2b. Mean Baseline Tone Burst ABR Latency (ms) for Left Ears in Male Rats

Frequency	I	II	III	IV	V
4 kHz	2.21	3.12	4.65	5.52	6.59
12 kHz	2.05	3.00	4.41	5.39	5.95
22 kHz	1.91	2.77	3.67	4.74	5.98
32 kHz	2.11	2.97	3.65	4.92	6.08

Table 3a. Mean 4 kHz ABR Latency (ms) for Right Ears in Male Rats

Condition	I	II	III	IV
Baseline	2.21	3.07	4.72	
Day 1	2.22	2.99	4.70	5.86
Day 14	2.08	2.92	4.45	5.88
Day 22	2.13	2.97	4.63	5.72

Table 3b. Mean 4 kHz ABR Latency (ms) for Left Ears in Male Rats

Condition	I	II	III	IV
Baseline	2.21	3.12	4.65	
Day 1	2.24	3.10	4.77	6.07
Day 14	2.20	3.06	4.77	5.15
Day 22	2.22	3.04	4.77	6.20

Table 4a. Mean 12 kHz ABR Latency (ms) for Right Ears in Male Rats

Condition	I	II	III	IV
Baseline	2.04	2.93	4.11	
Day 1	2.07	2.94	3.82	4.84
Day 14	1.97	2.86	3.51	4.58
Day 22	1.98	2.86	3.69	3.69

Table 4b. Mean 12 kHz ABR Latency (ms) for Left Ears in Male Rats

Condition	I	II	III	IV
Baseline	2.05	3.00	4.41	
Day 1	2.09	3.01	4.28	5.26
Day 14	1.97	2.83	3.60	4.69
Day 22	2.01	2.83	3.77	4.63

Table 5a. Mean 22 kHz ABR Latency (ms) for Right Ears in Male Rats

Condition	I	II	III	IV	V
Baseline	1.77	2.65	3.56	4.35	5.62
Day 1	1.79	2.65	3.56	4.40	5.7
Day 14	1.76	2.64	3.52	4.51	5.52
Day 22	1.72	2.61	3.31	4.27	5.44

Table 5b. Mean 22 kHz ABR Latency (ms) for Left Ears in Male Rats

Condition	I	II	III	IV	V
Baseline	1.91	2.77	3.67	4.74	5.98
Day 1	2.05	2.90	3.76		5.20
Day 14	1.91	2.75	3.63	4.44	5.79
Day 22	1.94	2.82	3.62	4.47	5.64

Table 6a. Mean 32 kHz ABR Latency (ms) for Right Ears in Male Rats

Condition	I	II	III	IV	V
Baseline	2.11	3.05	3.75	5.00	6.09
Day 1	2.25	3.09	3.87	5.20	6.46
Day 14	2.39	2.99	3.66	4.78	5.99
Day 22	2.18	3.02	3.66	4.90	6.04

Table 6b. Mean 32 kHz ABR Latency (ms) for Left Ears in Male Rats

Condition	I	II	III	IV	V
Baseline	2.11	2.97	3.65	4.92	6.08
Day 1	2.27	3.11	3.94	5.19	6.26
Day 14	2.12	2.97	3.60	4.77	6.12
Day 22	2.18	3.01	3.66	4.85	5.97

Table 7a. Mean Baseline ABR Latency (ms) for Right Ears in Female Rats

Freqeuncy	I	II	III	IV	V
4 kHz	2.16	3.00	4.77	6.04	
12 kHz	1.98	2.84	3.69	4.72	6.06
22 kHz	1.76	2.65	3.60	4.40	5.71
32 kHz	2.16	3.06	5.06	5.13	6.28

Table 7b. Mean Baseline ABR Latency (ms) for Left Ears in Female Rats

Freqeuncy	I	II	III	IV	V
4 kHz	2.27	3.12	4.83	6.15	
12 kHz	2.05	2.96	4.03	5.15	6.03
22 kHz	1.96	2.79	3.92	4.51	6.02
32 kHz	2.14	3.06	3.77	3.79	6.28

.

Table 8a. Mean 4 kHz ABR Latency (ms) for Right Ears in Female Rats

Condition	I	II	III	IV
Baseline	2.16	3.00	4.77	6.04
Day 1	2.13	2.98	4.62	5.77
Day 14	2.17	3.01	4.70	5.85
Day 22	2.12	2.84	4.67	5.85

Table 8b. Mean 4 kHz ABR Latency (ms) for Left Ears in Female Rats

Condition	I	II	III	IV
Baseline	2.27	3.12	4.83	6.15
Day 1	2.21	3.12	4.60	5.65
Day 14	2.23	3.14	4.85	6.10
Day 22	2.30	3.18	4.90	5.98

Table 9a. Mean 12 kHz ABR Latency (ms) for Right Ears in Female Rats

Condition	I	II	III	IV	V
Baseline	1.98	2.84	3.69	4.72	6.06
Day 1	1.99	2.87	3.83	4.63	5.90
Day 14	2.00	2.85	3.64	4.68	5.78
Day 22	1.98	2.84	3.76	4.96	6.20

Table 9b. Mean 12 kHz ABR Latency (ms) for Left Ears in Female Rats

Condition	I	II	III	IV	V
Baseline	2.05	2.96	4.03	5.15	6.03
Day 1	2.00	2.87	3.75	4.90	5.70
Day 14	1.98	2.85	3.83	4.96	5.83
Day 22	2.08	2.91	3.64	4.71	5.64

Table 10a. Mean 22 kHz ABR Latency (ms) for Right Ears in Female Rats

Condition	I	II	III	IV	V
Baseline	1.76	2.65	3.60	4.40	5.71
Day 1	1.79	2.64	3.54	4.32	5.60
Day 14	1.78	2.67	3.57	4.36	5.71
Day 22	1.78	2.65	3.54	4.24	5.60

Table 10b. Mean 22 kHz ABR Latency (ms) for Left Ears in Female Rats

Condition	I	II	III	IV	V
Baseline	1.96	2.79	3.92	4.51	6.02
Day 1	2.01	2.84	3.90	4.76	5.72
Day 14	1.97	2.79	3.71	4.69	5.84
Day 22	1.93	2.73	3.69	3.55	5.74

Table 11a. Mean 32 kHz ABR Latency (ms) for Right Ears in Female Rats

Condition	I	II	III	IV	V
Baseline	2.16	3.06	3.79	5.13	6.28
Day 1	2.28	3.14	3.79	5.01	6.07
Day 14	2.20	3.02	3.69	5.06	6.18
Day 22	2.22	3.04	3.67	4.97	6.07

Table 11b. Mean 32 kHz ABR Latency (ms) for Left Ears in Female Rats

Condition	I	II	III	IV	V
Baseline	2.14	3.06	3.77	5.06	6.28
Day 1	2.22	3.03	3.73	4.94	6.05
Day 14	2.16	2.94	3.56	4.54	6.18
Day 22	2.10	2.90	3.59	4.81	6.12

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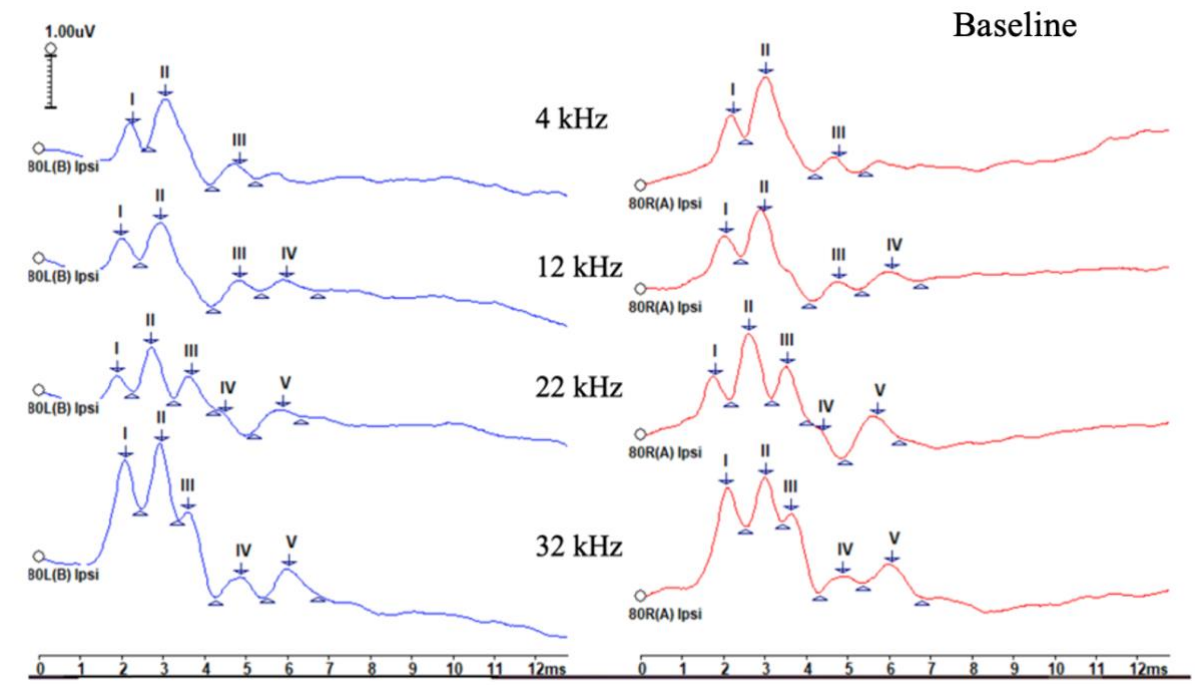

Figure 5. Average male tone burst ABR baseline (pre-migraine) at suprathreshold 80 dB SPL at 4, 12, 22, and 32 kHz for the left ear (blue; N = 8) and right ear (red; N = 8).

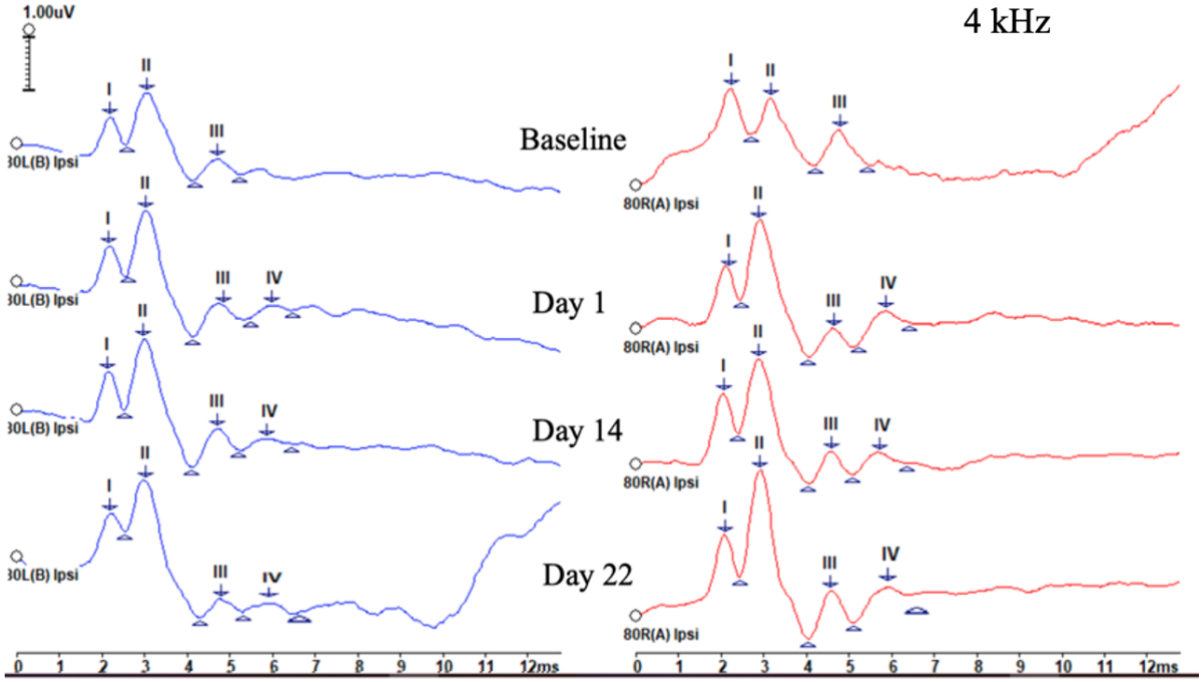

Figure 6. Average male tone burst, 4 kHz ABR at suprathreshold 80 dB SPL at baseline and and post migraine (day 1, day 14, and day 22) for the left ear (blue) and right ear (red).

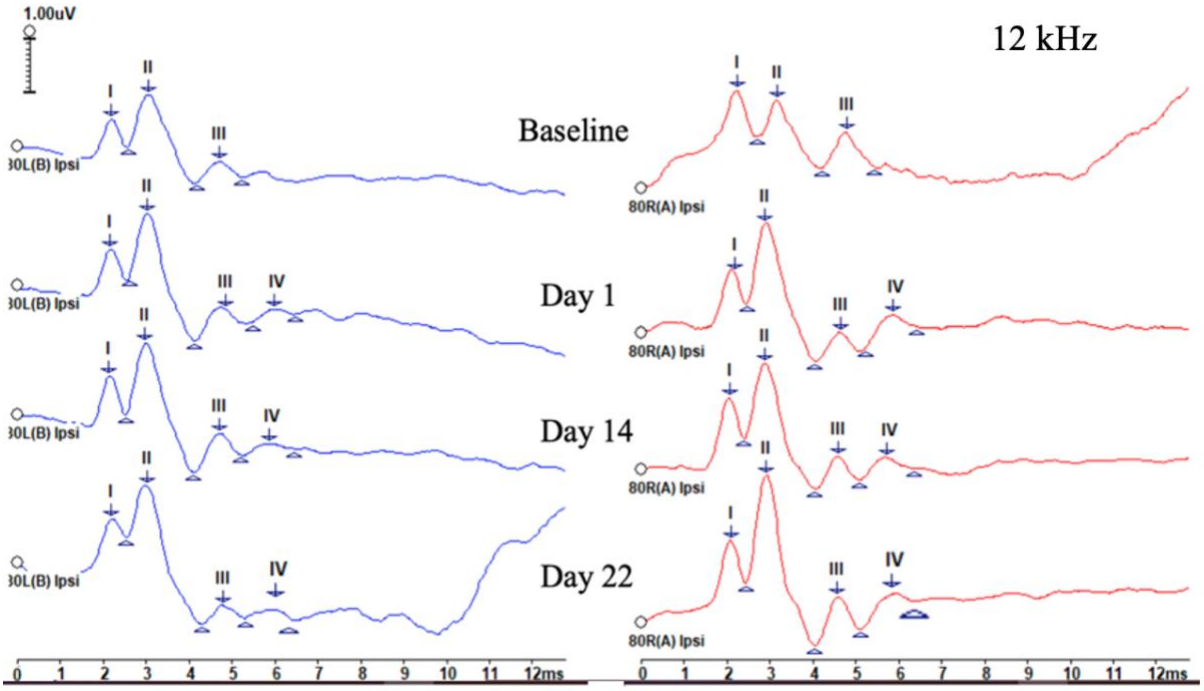

Figure 7. Average male tone burst, 12 kHz ABR at suprathreshold 80 dB SPL at baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 8) and right ear (red; N = 8).

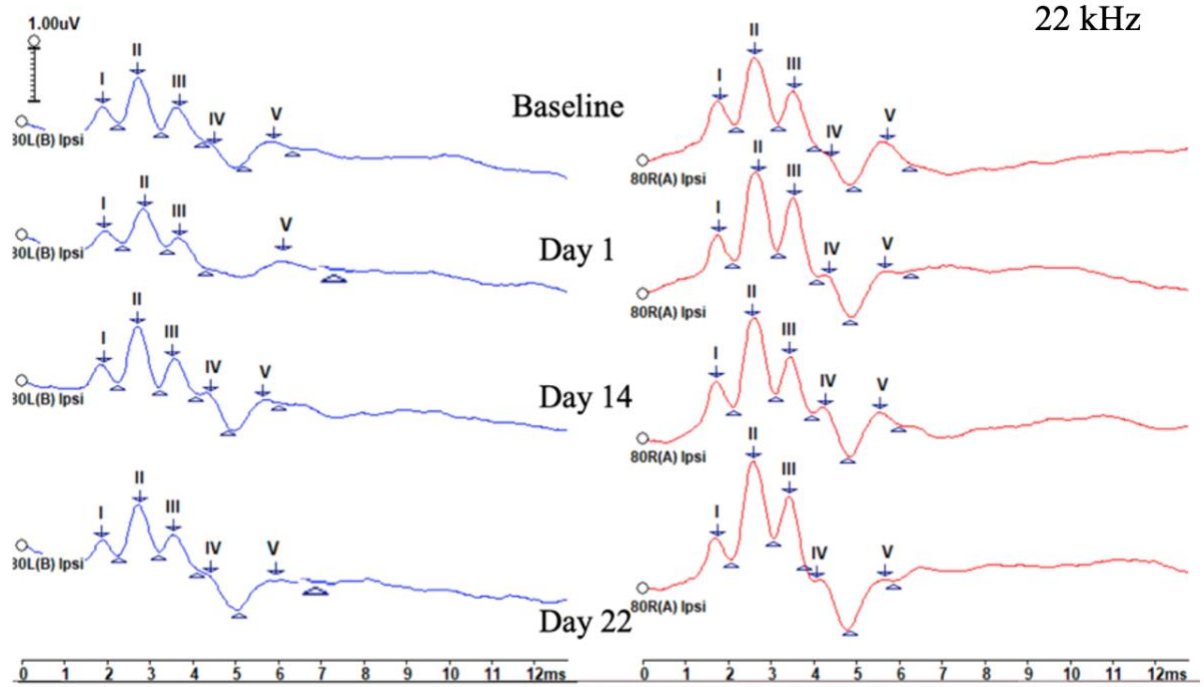

Figure 8. Average male tone burst, 22 kHz ABR at suprathreshold 80 dB SPL at baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 8) and right ear (red; N = 8).

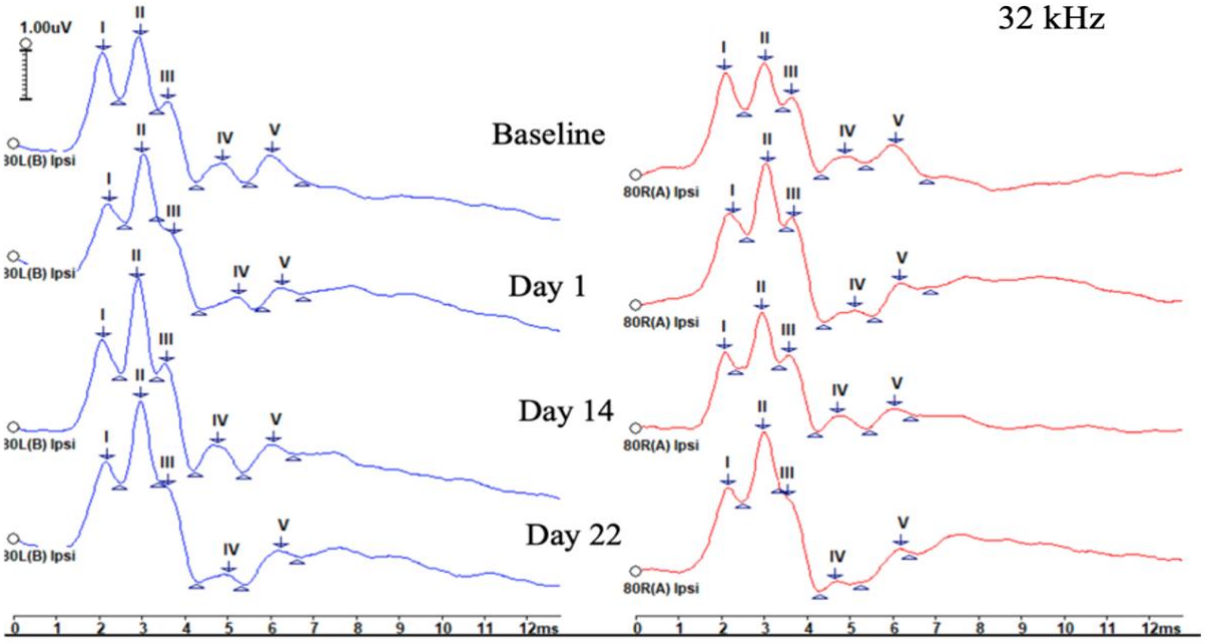

Figure 9. Average male tone burst, 32 kHz ABR at suprathreshold 80 dB SPL at baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 8) and right ear (red; N = 8).

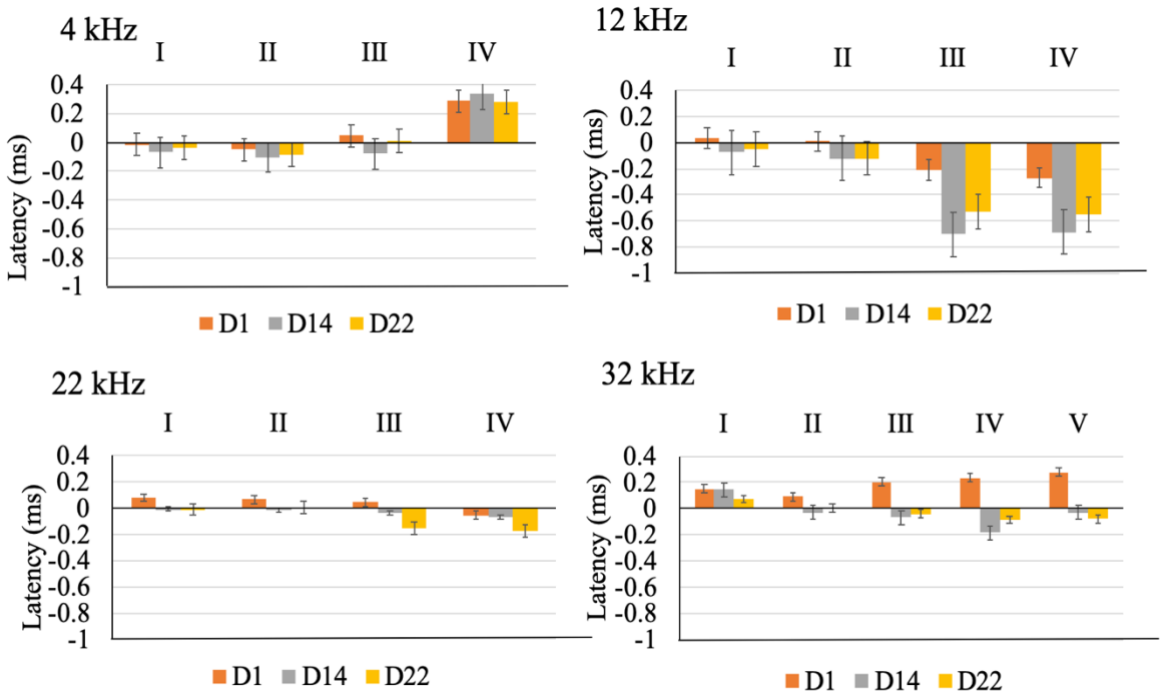

Figure 10. Male mean change (+/- SEM) in latency per frequency post migraine compared to baseline.. Male rats had the largest change in latency at 12 kHz. At 12 kHz, latency decreased post-migraine.

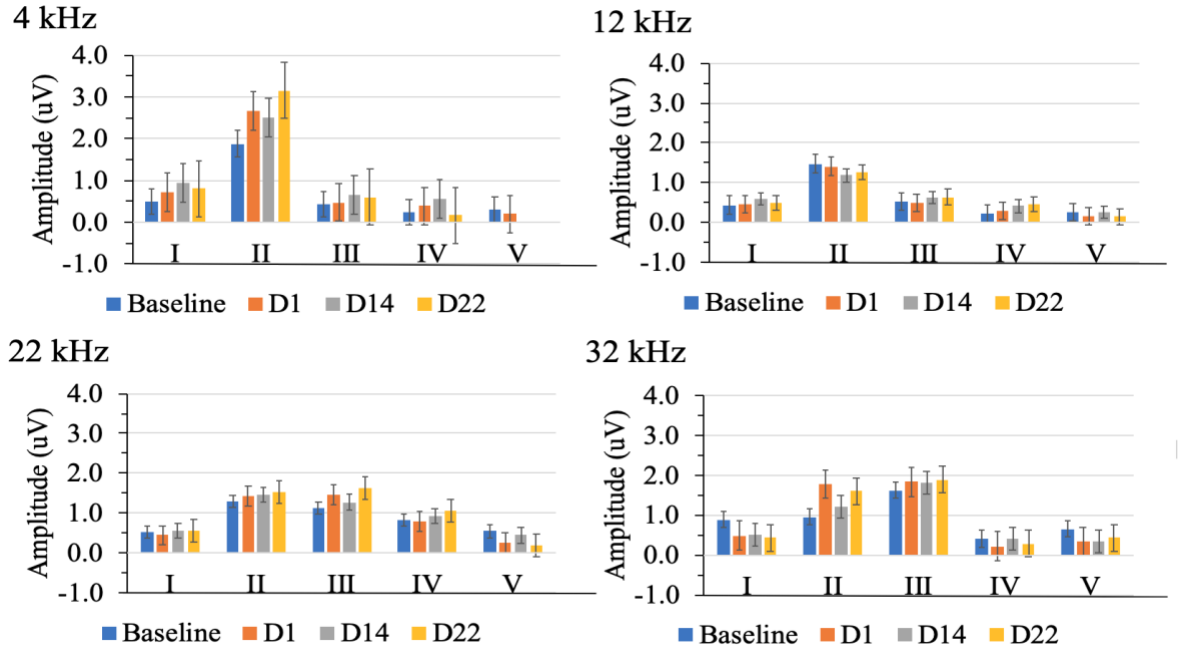

Figure 11. Male mean (+/- SEM) amplitude (μV) changes in the right ear at all four tested frequencies at baseline and changes post migraine (day 1, day 14, and day 22) in male rats. The largest amplitude change (enhancement) for male rats in the right ear was for wave II at 4 kHz.

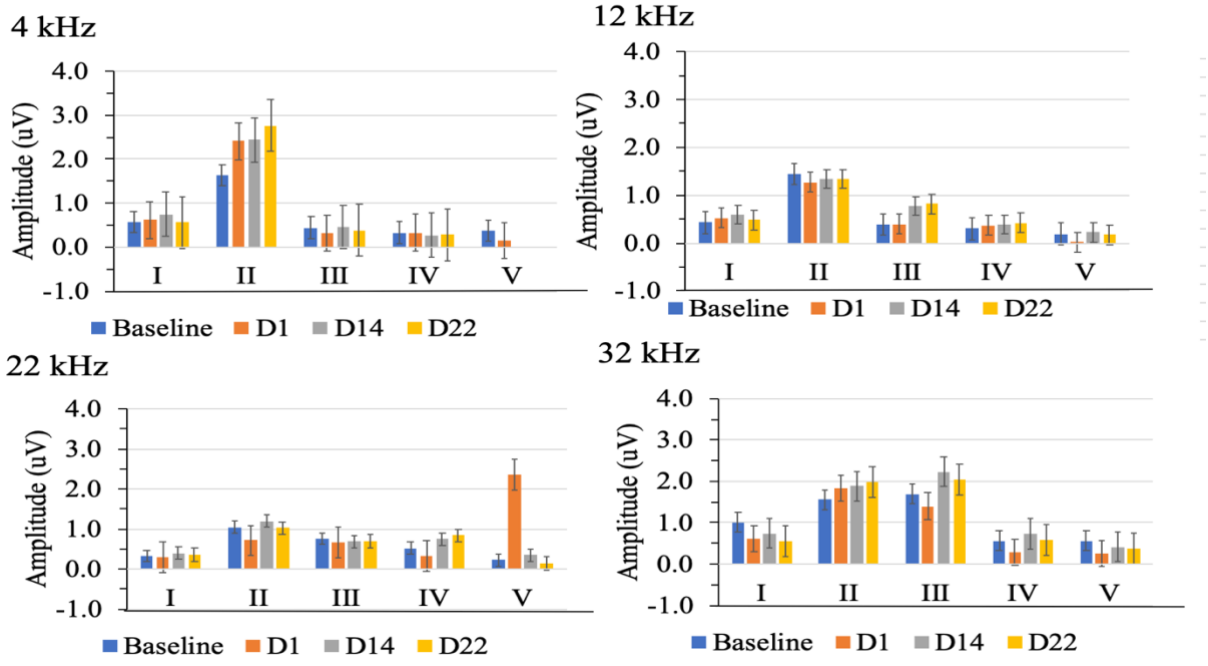

Figure 12. Male mean (+/- SEM) amplitude (μV) changes in the left ear at all four tested frequencies at baseline and changes post migraine (day 1, day 14, and day 22) in male rats. The largest amplitude change for male rats in the left ear was for wave II at 4 kHz.

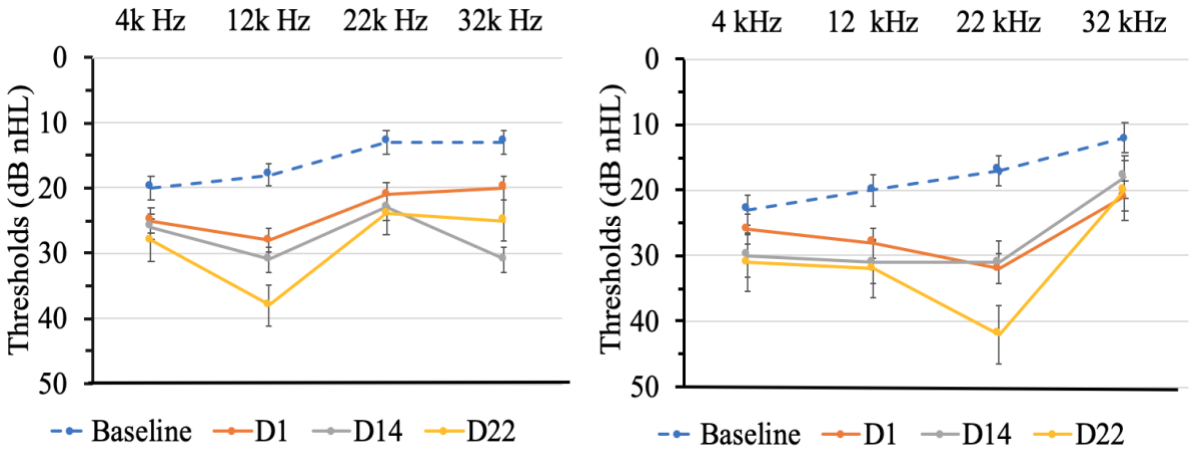

Figure 13. Male mean (+/- SEM) ABR thresholds at baseline, day 1, 14, and 22 post migraine at 4, 12, 22, and 32 kHz for the right and left ear. Both ears shows a consistent increase (worsening) in hearing thresholds throughout the course of the migraine. The right ear showed the most change at 12 kHz and the left ear showed the most change at 22 kHz, with worsening of hearing mainly at these two frequencies at day 22 post migraine.

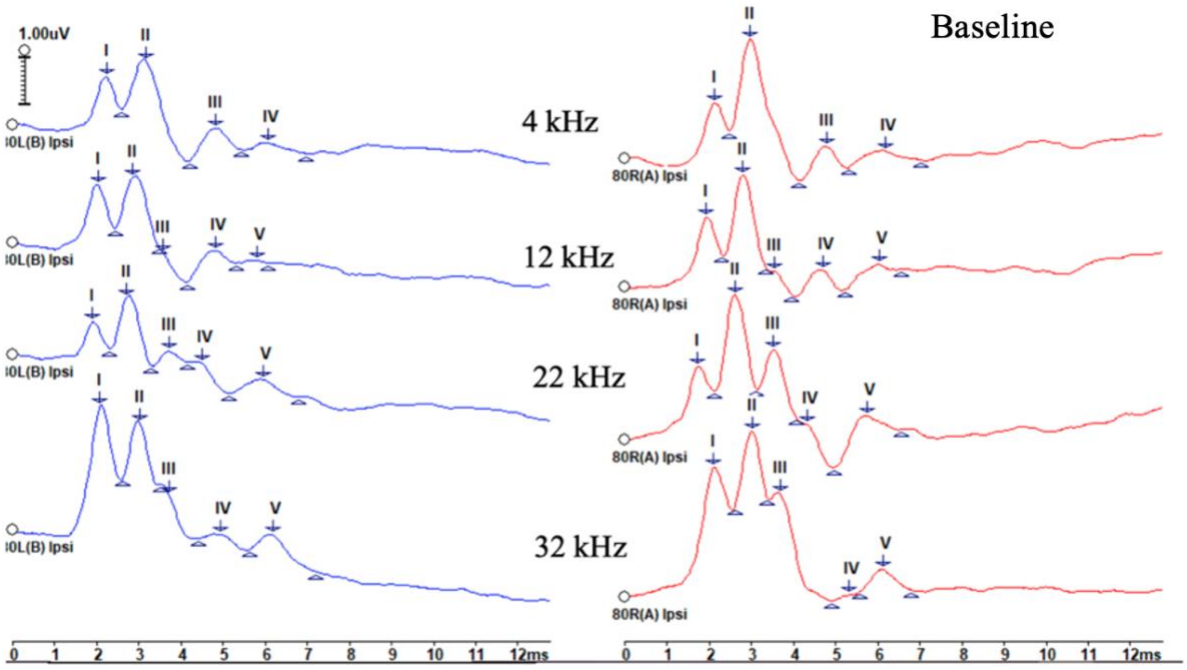

Figure 14. Average female tone burst ABR baseline (pre-migraine) at suprathreshold 80 dB SPLat 4, 12, 22, and 32 kHz for the left ear (blue; N = 6) and right ear (red; N = 6).

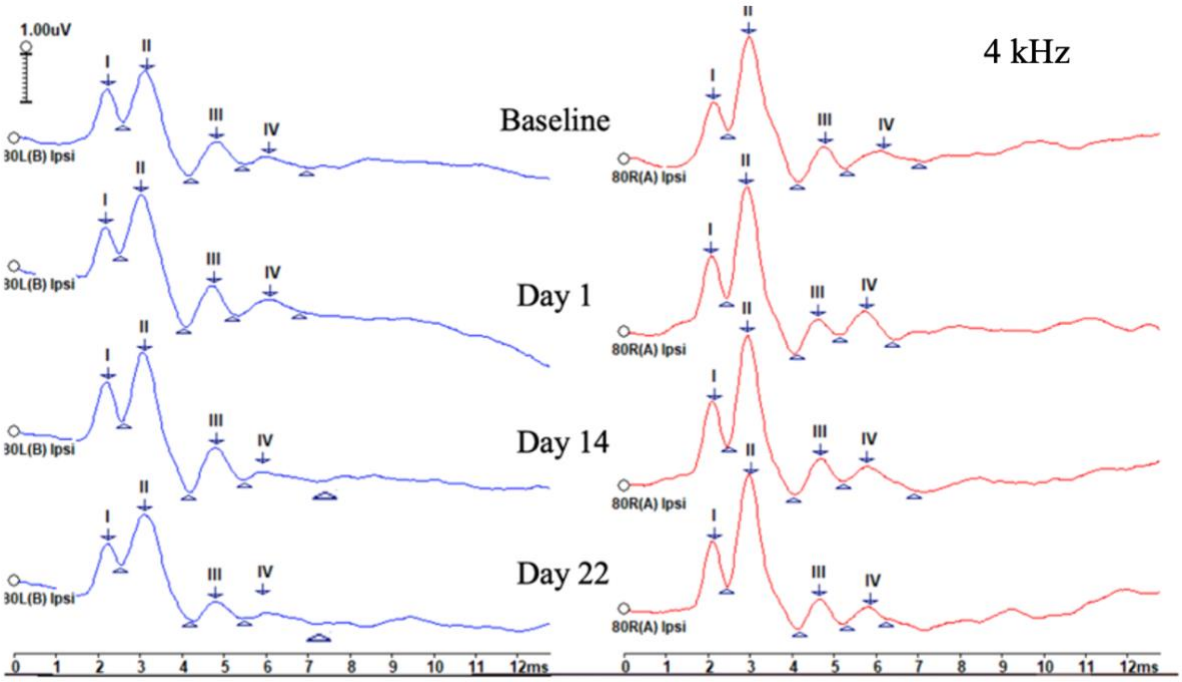

Figure 15. Average female tone burst, 4 kHz ABR at suprathreshold 80 dB SPL at baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 6) and right ear (red; N = 6).

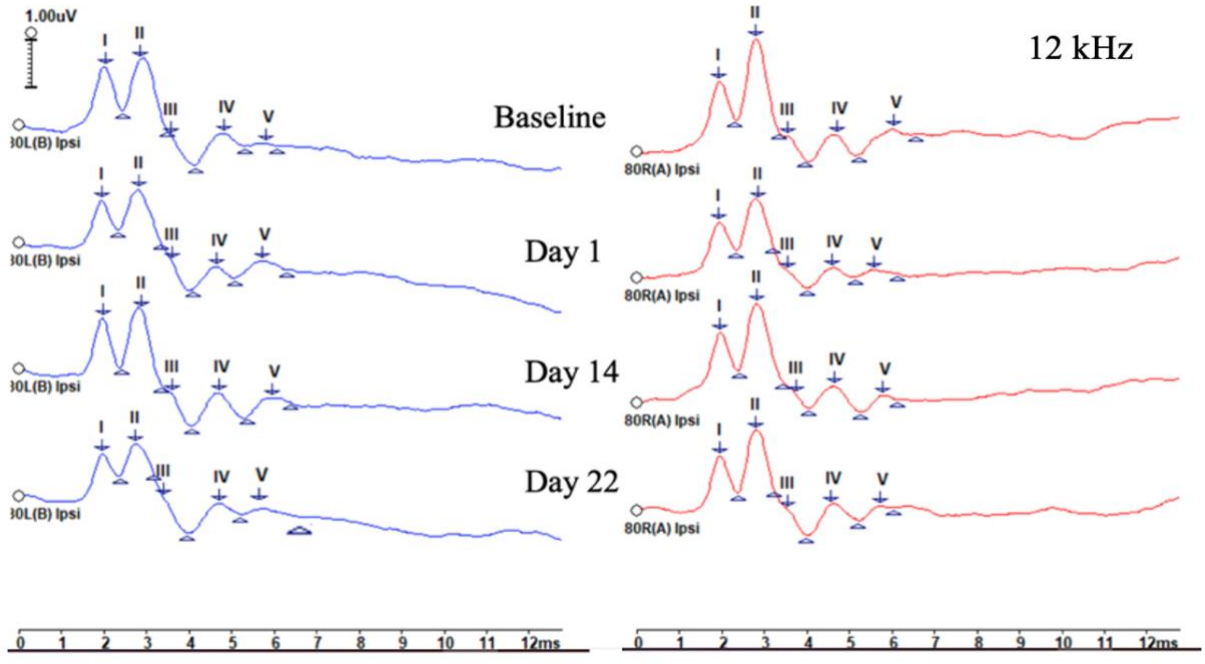

Figure 16. Average female tone burst, 12 kHz ABR at suprathreshold 80 dB SPL at baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 6) and right ear (red; N = 6).

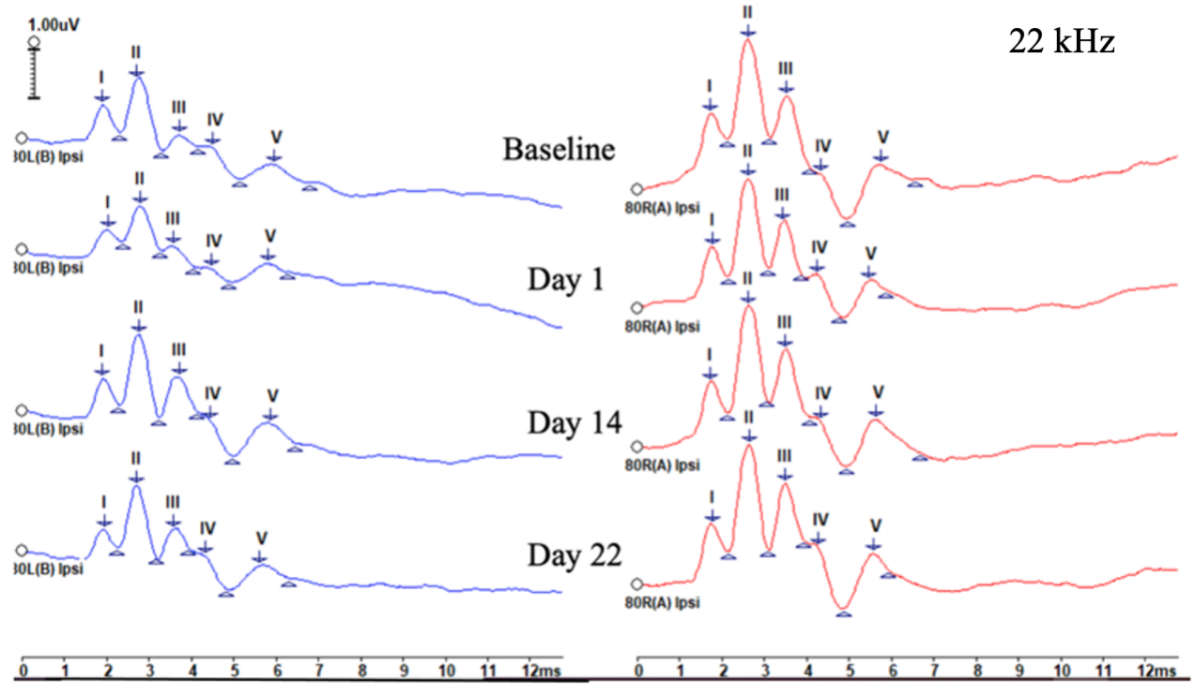

Figure 17. Average female tone burst, 22 kHz ABR at suprathreshold 80 dB SPL baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 6) and right ear (red; N = 6)

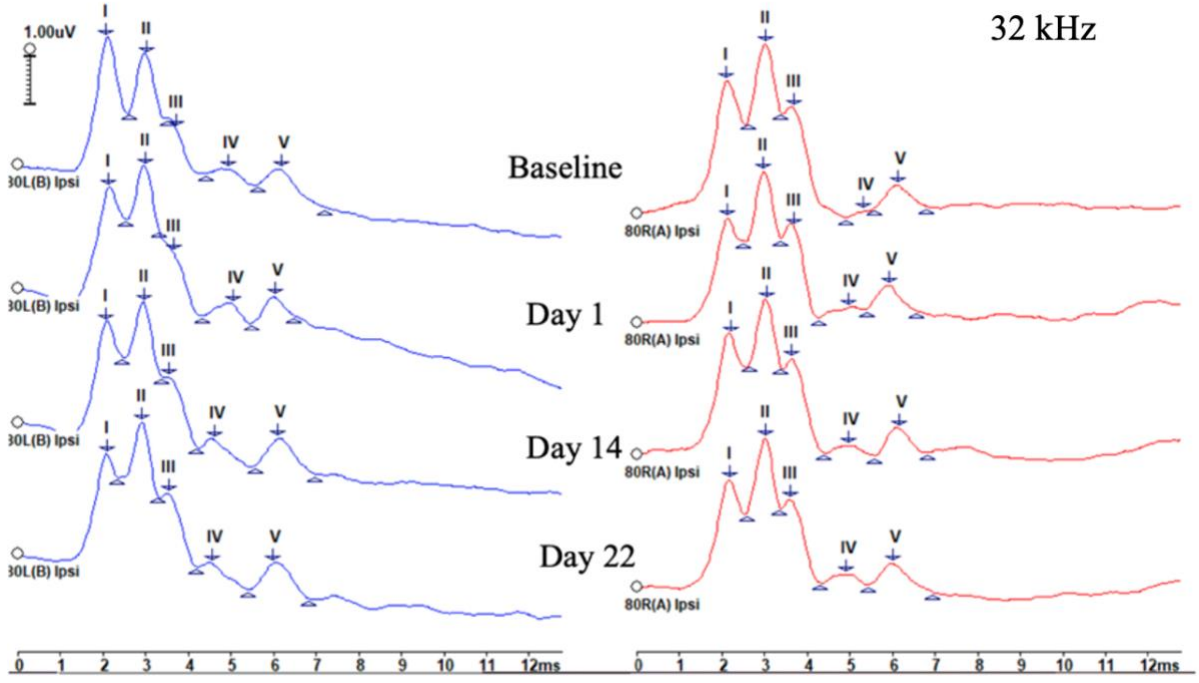

Figure 18**.** Average female tone burst, 32 kHz ABR at suprathreshold 80 dB SPL baseline and post migraine (day 1, day 14, and day 22) for the left ear (blue; N = 6) and right ear (red; N = 6).

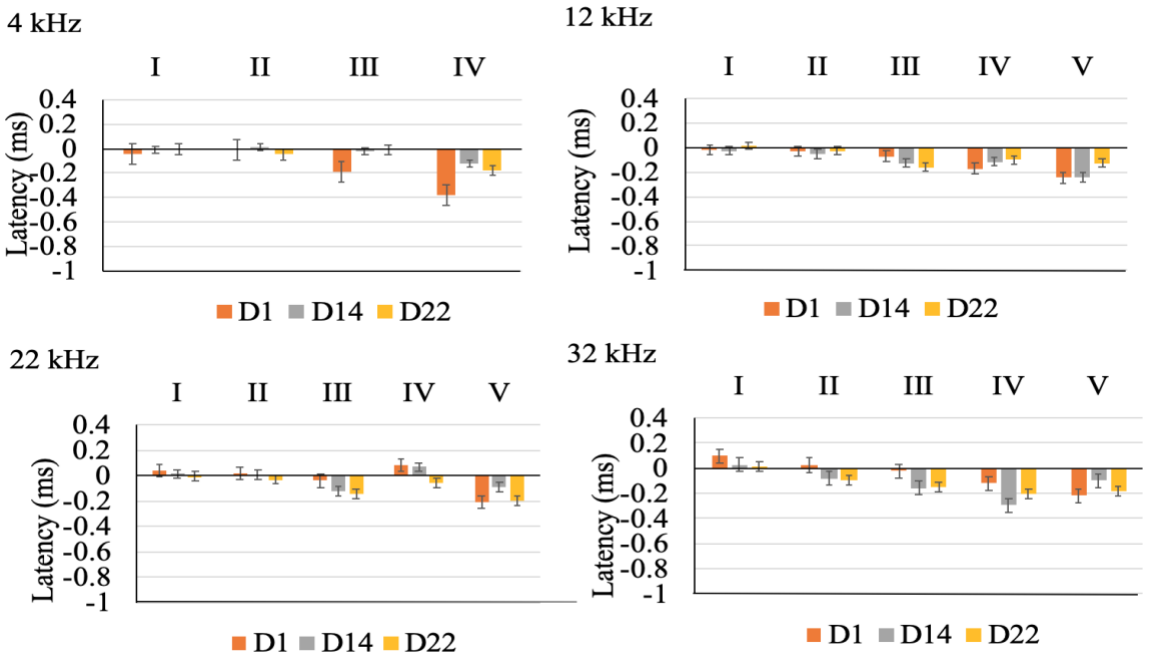

Figure 19. Female mean (+/- SEM) change in latency per frequency post migraine compared to baseline. All frequenices showed an increase in latency post-migraine.

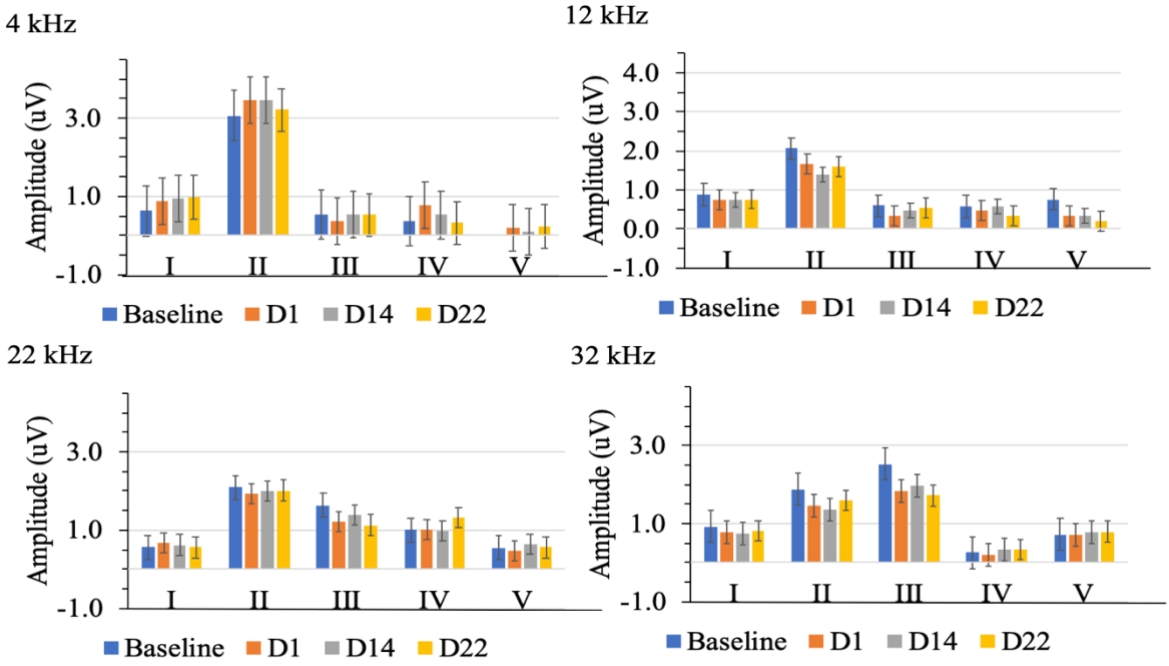

Figure 20. Female mean (+/- SEM) amplitude (µV) changes in the right ear at all four freqeucnies in female rats at baseline and changes post migraine (day 1, day 14, and day 22). The largest amplitude change for female rats in the right ear was for wave II at 4 kHz.

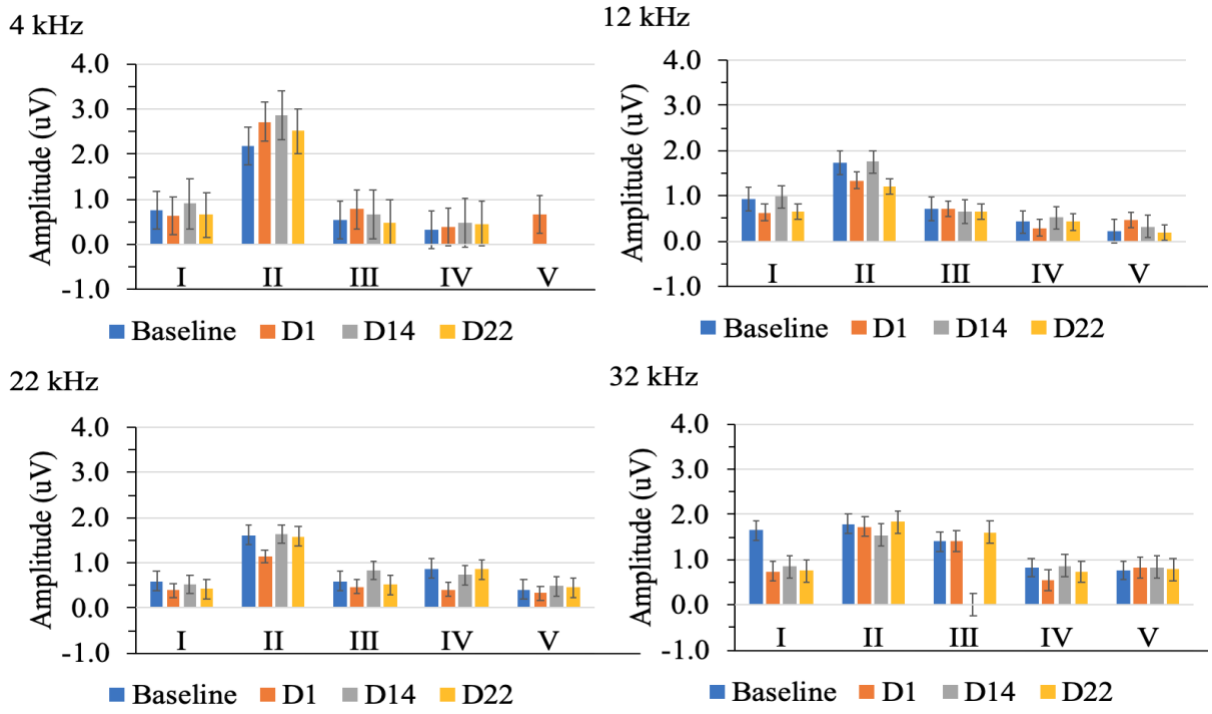

Figure 21. Female mean (+/- SEM) amplitude (µV) changes in the left ear at all four freqeucnies at baseline and changes post migraine (day 1, day 14, and day 22) in female rats. The largest amplitude change for females rats in the left ear was for wave II at 4 kHz.

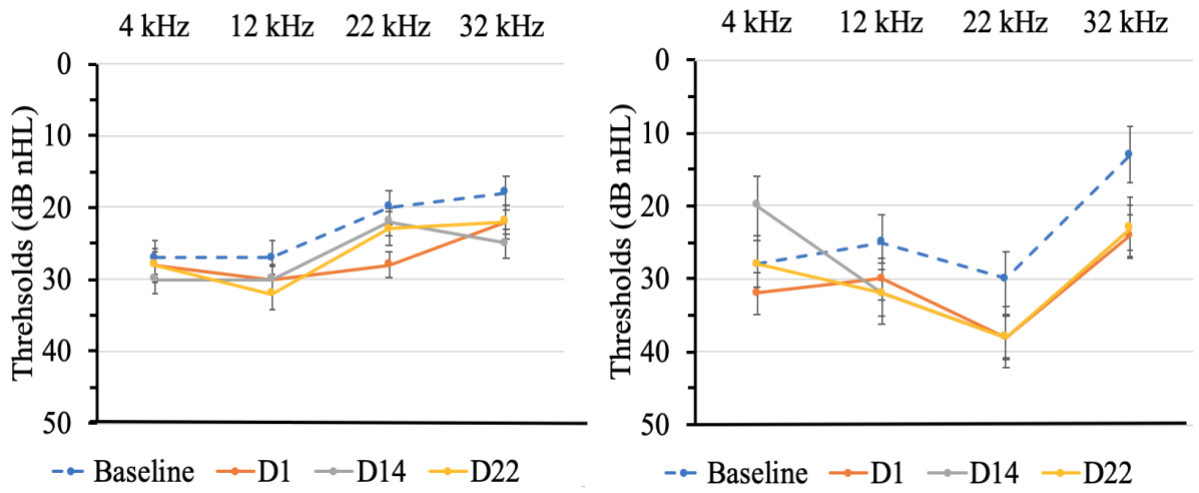

Figure 22. Female mean (+/- SEM) ABR thresholds at baseline, day 1, 14, and 22 post migraine at 4, 12, 22, and 32 kHz for the right and left ear. Both ears shows a consistent increase (worsening) in hearing thresholds throughout the course of the migraine.

DISCUSSION

Goal of the Study

This goal of this study was to investigate changes in the auditory brainstem response and hearing threshold in an experimentally induced chronic migraine state characterized by sustained nocifensive hypersensitivity to mechanical stimulation in the orofacial region [27]. This is an established preclinical chronic migraine model that involves prolonged sensitization of trigeminal neurons mediated by neck muscle inflammation and one night of REM sleep deprivation. Subjecting animals to these two known migraine risk factors, neck muscle tension and sleep deprivation [30], is sufficient to lower the activation threshold of trigeminal neurons to a pungent odor from the CBL tree, which is also known as the headache tree [28]. These leaves contain a compound known as umbellulone that causes activation of the trigeminal nerves and subsequent release of the neuropeptide calcitonin gene-related peptide (CGRP) leading to enhanced pain signaling [28, 31]. In this model, exposure of sensitized male and female animals to the pungent odor promotes a sustained state of trigeminal sensitization characteristic of the persistent neuronal hyperexcitability reported in chronic migraineurs [27]. Enhanced mechanical nociception was observed on day one following exposure to the pungent odor and this level of heightened sensitivity was maintained for several weeks. Hence, this model was used to investigate changes in the latency and amplitude of ABR waveforms, and hearing threshold at several frequencies since migraine patients report that sound sensitivity is one of their most bothersome symptoms [8, 10, 32].

Summary of Thesis

In this novel study, the chronic migraine model was established using eight male and six female Sprague Dawley rats since it was not known if sex differences would be observed in the auditory brainstem system in response to migraine pathology, which is thought to originate in the central nervous system. To assess neural changes in the brainstem, suprathreshold tone burst ABRs were recorded at 80 dB SPL at 4 kHz, 12 kHz, 22 kHz, and 32 kHz at baseline. ABR waveform morphology, latency, and amplitude responses were analyzed. A chronic migraine state was then induced in the rats using neck muscle tension, REM sleep deprivation, and exposure to a pungent odor. ABRs were then rerecorded on day 1, 14, and 22 after induction of the chronic migraine phenotype. ABR morphology, latency, and amplitude was then compared pre and post migraine state to study any changes that might be mediated in the rat's auditory system. To determine the threshold, tone burst ABR was recorded at 50 dB and 20 dB and then at 30 dB or 10 dB depending on the response at 20 dB. The threshold value was defined as the lowest intensity to elicit a reliable wave II, which is the largest wave in rats. At baseline (naïve condition), the 80 dB SPL ABR morphology exhibited the presence of distinct waves I, II, and III at lower frequencies (4 kHz and 12 kHz) and waves I, II, III, IV, and V at higher frequencies (22 kHz and 32 kHz), with wave II being the largest wave at all frequencies as expected. As the frequencies increased from 4 kHz to 32 kHz, latencies decreased, and amplitudes increased with a larger amplitude observed in the right ear (wave II mean = 1.98 μV) compared to the left ear (wave II mean = 1.65 μV) (are these differences statistically significant?). Thresholds were within normal range, with better ABR thresholds at higher frequencies (mean =13 dB SPL) than lower frequencies (mean = 20 dB SPL). After induction of migraine pathology, changes were observed including mild-moderate hearing loss, decreased latency at 4 kHz, 12 kHz, 22 kHz, and

32 kHz, and increased amplitudes, mainly observed in wave II, at 4 kHz and 32 kHz. Enhancement of amplitude occurred in both the left and right ear post migraine, however, 22 kHz showed an ear difference where the right ear had larger enhancement of ABR waves and earlier latencies compared to the left ear in both male and female rats. Results from this novel study provide, to our knowledge, the first evidence of profile changes in brainstem response amplitude and latency and high frequency hearing sensitivity in a preclinical chronic migraine model. The presence of earlier response latency and larger amplitudes post-migraine suggest ␣␣, a finding in agreement with patients suffering from chronic migraine [8]. The presence of hearing loss post migraine, most probably is cochlear in origin, is believed to be due to interruption of blood flow of inner ear [33].

Male vs Female

Overall, there were only minor differences found between the male and the female or between left and right ear ABR data pre or post induction of chronic migraine pathology. For example, latency averages for males and females were overall similar. Wave II latency for males and females were within 0.09 ms of each other. Further, males had larger amplitudes at 4 kHz and decreased amplitudes at 12 kHz and compared to females. An observed difference was that males had more pronounced morphology changes in their ABR compared to females throughout the course of the migraine. In addition, males had better ABR thresholds at all frequencies compared to females. The average male threshold was 17 dB SPL, while the average female threshold was 23 dB SPL. Few comparative studies of sex and ABR have been published but one study reported significant sex differences in ABRs thresholds and found that males had worse thresholds at 1 kHz, 4 kHz, 32 kHz, and 42 kHz compared to females[34]. However, Sprague

Dawley rats were not used in their study. Since differences are likely to be related to the rodent species given differences in their nervous systems and vigilance levels, more studies are needed to determine threshold differences between male and female rats and determine which species may best mimic pathological conditions reported in humans.

Migraine and Auditory Hypersensitivity

Auditory dysfunction associated with migraine is understudied, especially given that heightened sensitivity to sounds is reported as a most bothersome symptom in migraineurs during an attack [8, 17, 18]. Tawfik et al., [35] reported that few studies have been done to explore the relationship between chronic migraine and auditory function. However, although few in number, data from those studies provide evidence of abnormalities in hearing thresholds and ABRs. Abnormalities in ABR indicate impending auditory malfunction in migraine and disruption of central auditory processing mechanisms [8, 36]. Therefore, this condition can predispose a migraine sufferer to be hypersensitive to normal sounds in one's everyday environment. Our findings of earlier ABR latency and enhanced response amplitudes post migraine are indications that migraine may result in hyperexcitability of the central auditory pathway and neural transmission at the brainstem level. This finding is consistent with the reported enhanced ABR waves II, III, and IV in migraineurs [8]. In addition, this hyperexcitability (or enhanced central gain) at the brainstem level could be a compensatory mechanism secondary to the presence of sensorineural hearing loss of cochlear origin. This is supported by the correlation between hearing loss and waves II and III enhanced amplitude response in migraineurs [8] however, when hearing loss is severe ABR response latency are expected to be delayed/prolonged and response amplitudes are small. Several studies have

indicated that enhanced central gain is a possible mechanism for hyperacusis and tinnitus [8, 15, 33]. Our results suggest that migraine is believed to cause both mild-to-moderate hearing loss and enhanced central gain, and thus may result in hyperacusis and tinnitus. Ashkenazi et al., [37] have reported that individuals with migraine have shown reduced tolerance to sound as tested by uncomfortable loudness level during migraine attacks, and recent findings revealed a very low uncomfortable loudness level (around 50 dB HL at 500 Hz to around 38 dB HL at 4000 Hz) (Putnam and Sims, unpublished work). This very low level of uncomfortable loudness is much lower than the mean level (76-78 dB HL) reported in individuals with hyperacusis [38]. This may be explained by a possible exacerbation of hyperacusis caused by enhanced light sensitivity, as another common symptom in migraineurs. Future studies are needed to investigate whether migraine causes enhanced central gain at more proximal central auditory pathway. Given that the emotional reaction to hyperacusis (and tinnitus) is triggered by the limbic system, mainly the hypothalamus and amygdala, examination of the hyperexcitability and histopathology examination of these structures in a preclinical migraine model may determine a possible pathophysiology of hyperacusis in migraine.

Recommendations

Sound hypersensitivity is such a strong and unpleasant symptom when dealing with chronic migraine, however, there are few validated recommendations for how to deal with the sound hypersensitivity. In a study done by Ishikawa et al., [39] everyday sounds were studied on how migraine patients perceive them as noise. It was found that ambulance/police car sirens, railroad crossing bells, cicadas, car horns, and bird sounds were the most discomforting for migraine patients compared to a control group. It is recommended that patients who suffer from

migraine wear earplugs to reduce environmental noise and wear noise cancelling headphones to reduce the amount of sensory input to the auditory system and to protect from painful noises. It is also recommended that patients move their bed to a quieter location to minimize outside noise and to move your desk away from any loud machinery at work. Patients can also use white noise to mask background noise and reduce the risk of noise-induced headaches, especially in crowded environments. Patients can also consider installing sound-absorbing materials such as acoustic tiles and carpet to help absorb and dampen sounds. Those migraine patients who suffer from sound sensitivity should also advocate for themselves and be comfortable asking others to turn down the volume of a TV or stereo if it becomes painful. Another concern is that most migraine studies rely on a questionnaire about hearing sensitivity and the use of the word phonophobia which may not accurately represent the auditory changes experienced by most migraineurs [11, 37, 40-42]. There is a need to better educate migraine clinicians about the difference between phonophobia and hyperacusis and audiologists about migraine, and design better clinical trials to address changes in the auditory system prior to, during, and post a migraine attack.

Limitations

There were a few limitations noted in the study. The first limitation was sample size. Because we used a small sample size of eight male and six female rats, variability was seen during the data collection process. If a larger sample size was used, the results would likely be more consistent and exhibit less variability in the individual measurements. The second limitation was not measuring the animals body temperature during the ABR recordings, which can influence the results. Each 0.5 degrees Celsius decrement of body temperature can significantly affect latency and amplitude of the ABR [24]. It is recommended that a heating pad

be used to maintain the animal's body temperature since body temperature can become lower when an animal is under anesthesia for a prolonged time. However, all testing was performed under the same temperature-controlled environmental conditions and the amount of time to gather the data was similar for all experimental conditions.

Future Studies

For future studies, it may be interesting to see if currently used anti-migraine therapies would also be effective in minimizing the changes observed in the auditory system in our chronic migraine model. Therapies of interest would be those that are approved for the prevention and treatment of chronic migraine including topiramate, amitriptyline, botulinum neurotoxin type A (Botox), gepants, and anti-CGRP biologicals [43, 44]. In support of this notion, in a study by Abouzari et al., [45], the authors reported that their results provided evidence that for some patients, hyperacusis may share a pathophysiologic basis with migraine disorder and may be successfully managed with multimodal migraine prophylaxis therapy. Recent advances in understanding the pathophysiology of migraine provide the potential to reduce the impact of migraine and associated auditory changes such as hyperacusis. With the ability to treat migraine, there is also the ability for patients to restore normal function and allow them to return to work, school, and family if they get relief from their headache and most bothersome symptoms including sensitivity to normal light and sounds. Also, it would be of interest to determine if animals with altered hearing or auditory processing, and likely enhanced sensitivity to external stimuli, would be more susceptible to migraine triggers such as strong odors. This is an important factor to consider since many migraine patients report hypersensitivity to sensory stimuli prior to and during a migraine attack.

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APPENDIX: MISSOURI STATE UNIVERSITY IACUC APPROVAL NOTICE

Missouri State
UNIVERSITY

March 1, 2023

RE: IACUC protocol 2021-18

Megan Huelsing,

IACUC protocol #2021-18 entitled "Investigating hearing changes in rat models of migraine and TMD" was approved by the committee on September 14, 2021, and expires September 13, 2024.

The protocol reflects that you are approved to work with Dr. Paul Durham on this project.

Thank you and if you need anything in the future regarding this protocol, please contact me either via email (johnnapedersen@missouristate.edu) or at 417-836-3737.

Sincerely,

Johnna Pedersen

Johnna Pedersen
IACUC Administrator/Member
Interim Director of Research Administration

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ANIMAL CARE & USE APPLICATION

INSTITUTIONAL ANIMAL CARE & USE COMMITTEE

v. July 2019

All Animal Care & Use Applications should be submitted electronically to IACUC@missouristate.edu.

A. Investigator Information		
Principal Investigator: Dr. Paul Durham	Department: Biology	Office Address: Temple Hall 225
Office Phone: 417-836-4869	Emergency Phone: 417-836-3026	Email: PaulDurham@MissouriState.edu

B. Project Information

Project Title: Investigating hearing changes in rat models of migraine and TMD

Protocol Action:	**Protocol Type:**	**Protocol Class:**
☒ New Proposal	☒ Research	☐ Agricultural
☐ Pilot Study	☐ Teaching	☐ Behavioral
☐ Renewal (due to protocol expiration)		☒ Biomedical
☐ Review for Exemption		☐ Wildlife/Conservation

Is this project externally funded and/or do you anticipate future funding? ☐ **Yes** ☒ **No**

If Yes, what is the name of the Funding Agency and grant number/title? N/A

C. Previous Approved Protocol

For work that is similar to a previously approved protocol, provide the original protocol number and approval date. On the remainder of the forms, indicate changes to the originally approved protocol in bold font.

Original Protocol Number:	Approval Date:
N/A	N/A

D. Investigator Assurances

1. The information provided herein is accurate and, to the best of my knowledge, conforms to all applicable University, PHS, and USDA policies on the use of animals in research and teaching.
2. I affirm that all procedures involving vertebrate animals will be performed only by personnel trained in the humane care, handling, and use of animals and that all personnel will abide by the recommendations of the University's Occupational Health and Safety Program.
3. I agree not to proceed with any portion of this project until I receive written approval from the Missouri State University Institutional Animal Care and Use Committee.
4. I agree any changes in the procedures contained in this protocol will be promptly forwarded to the IACUC for review and approval prior to performing any revised procedures.
5. I agree to provide proper, current documentation (e.g., licenses, permits and additional approval forms), when applicable, to the Office of Research Compliance throughout the course of this project.
6. I agree to allow inspection of my research facilities by members of the IACUC, including the veterinarian, and to comply promptly if informed of any violations of the Missouri State University's Animal Care and Use Policy.
7. I have taken into consideration the three "Rs" (replacement, reduction, and refinement) for my study and provided adequate justification for the animal model chosen, animal numbers requested, and procedures to reduce pain and distress.
8. I have conducted a literature search to ensure that I am not unnecessarily duplicating previous experiments.

Paul L. Durham	8/27/2021
Signature of Principal Investigator	Date