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Refining the procedures for medial-olivocochlear reflex (MOCR) assays: decreasing the contralateral noise reset time below 10 seconds can affect subsequent transient-evoked otoacoustic emission (TEOAE) measurements

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ABSTRACT

The medial-olivocochlear reflex (MOCR) may indicate risk for noise-induced hearing loss, but human clinical trials are needed. The transient-evoked otoacoustic emission (TEOAE)-based assay we have developed for assessing the MOCR needs to be faster for clinical use. This assay uses a series of TEOAE tests with and without a contralateral elicitor, and the MOCR strength is derived from their difference. This experiment evaluated if the elicitor reset time, which allows time for the MOCR to recover before the next TEOAE test, could be reduced from 10 seconds without affecting the test results. Four reset times (1.5, 3, 5, and 10 seconds) were compared for three transient-evoked otoacoustic emission (TEOAE) stimulus levels: 47, 50, and 53 dB SPL. All three shorter reset times showed residual effects on the following TEOAE response in some ears. It was unclear to what extent this affected the derived MOCR as no consistent pattern was found. We recommend that the 10 second reset time is retained for now.

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Contents

Figures

Tables

Executive Summary

The medial-olivocochlear reflex (MOCR) may indicate risk for noise-induced hearing loss, but human clinical trials are needed. The transient-evoked otoacoustic emission (TEOAE)-based assay we have developed for assessing the MOCR needs to be faster for clinical use. This assay relies on a contralateral elicitor (Marshall et al., 2014), which is typically a broadband noise. An OAE test in the ipsilateral ear is first conducted without the elicitor (a Q trial) and repeated with the elicitor (an N trial). The MOCR strength is calculated from the normalized difference in the OAE level between the Q and N trial. Typically, a series of Q-N trial-pairs are measured to ensure measurement reliability and stability. In an N trial, the elicitor is turned on two seconds before the OAE test to allow the MOCR to react to the elicitor and to reach a steady-state before beginning the OAE test. After the OAE test in the N trial is completed, there is a delay for 10 seconds before starting the next Q trial OAE test. This delay (the "reset" time) allows the MOCR to recover from any residual effects of the elicitor and to reach a steady state. For a clinical assay, it would be preferable to reduce the recommended 10 second reset time.

This small-scale (10 ears) experiment developed methods to assess the impact of reset time. Three reset times were evaluated (1.5, 3, and 5 seconds) and compared to the default 10 seconds, in the same ears. Also evaluated were three transient-evoked OAE (TEOAE) stimulus levels: 47, 50, and 53 dB SPL. A series of approximately six Q-N trial-pairs were collected in each ear for each condition. The first trial of the series was a Q trial (Q1). It did not have a preceding N trial, so Q1 could not have been affected by contralateral elicitor activity. Q1 was compared to a prediction interval calculated from the approximately five subsequent Q trials (Qrest), which may have been affected by residual elicitor activity. Evidence was found that even up to five seconds after the elicitor stopped, the TEOAE level in the next Q trial could be affected by the preceding elicitor. Similar calculations were made for the normalized MOCR calculated from each Q-N trial pair, but no clear pattern was found. We recommend that the 10-second reset interval is retained when using the TEOAE-based MOCR assay, as described in Marshall et al. (2014), until more definitive evidence of its effects is shown.

1 Introduction

The otoacoustic emission (OAE)-based clinical assay of the medial olivocochlear reflex (MOCR) developed at the Naval Submarine Medical Research Laboratory (NSMRL), (Marshall et al., 2014), is a test of a protective reflex thought to reduce the damage and hearing loss associated with loud noises (summarized in Marshall & Lapsley Miller, 2015). The MOCR assay takes around nine minutes to run per ear, assuming three trial-pairs are measured to assure measurement stability. Anecdotal reports from potential end users (such as military audiologists running hearing conservation programs, HCPs) has indicated that this test time is too long for a clinical assay. In a typical HCP, multi-person batch testing is done in approximately 15-20 minute blocks where other testing must also occur, including the pure-tone audiometry hearing test. The lengthy MOCR assay also restricts how many trial-pairs can be measured in a typical laboratory session where many trial-pairs are measured to build a distribution of intrasubject variability. We are investigating multiple ways to reduce this test time. In this report, we consider if the reset time may be reduced.

The MOCR assay relies on a broadband noise contralateral elicitor (Marshall et al., 2014). During a series of ipsilateral OAE tests, a contralateral MOCR elicitor is cycled on and off. Trials without an elicitor are termed "Q" or quiet trials, and trials with an elicitor are termed "N" or noise trials. Trial-pairs consist of contiguous Q and N trials. The normalized MOCR strength (MOCR%) is defined as the difference in OAE level in a trial-pair, which is calculated by subtracting the complex OAE level in the N trial from the OAE level in the Q trial and normalizing by the Q trial amplitude. Typically, during the N trial, two seconds before the OAE test begins, the elicitor is turned on to allow the MOCR to reach a steady-state (Backus & Guinan, 2006) before beginning the OAE test. After the N-trial OAE is measured, there is a 10-second delay before starting the next OAE test, which is a Q trial. This delay allows the MOCR to fully decay (Backus & Guinan, 2006) so that there are no residual effects of the elicitor. If the MOCR has not fully decayed to its baseline steady state at the end of the reset time after an N trial, the TEOAE may be affected in the following Q trial, which would affect the derived MOCR%.

The ten-second reset time used in earlier research (Marshall et al., 2014) is a conservative estimate, on advice from Dr. John Guinan (Harvard Medical School, personal communication, 2003). His recommendations were based on his research into the MOCR time-course and how he believed these results would apply to our proposed assay. These estimates came from laboratory studies using a different MOCR method where the time course was modeled as sums of complex exponentials, each with its own time constant (Backus & Guinan, 2006). When the MOC elicitor is turned on, the MOCR% rises exponentially to reach a steady state. When the elicitor is turned off, the MOCR% decays exponentially to reach the baseline state. The length of time it takes to reach these steady states was described as being due to a neural delay, a medial-olivocochlear to outer-hair cell synaptic delay, and a reverse otoacoustic delay (Backus & Guinan, 2006). The authors caution that the delays measured may be dependent on the specific stimuli used.

Unlike their method, our TEOAE-based assay does not provide the time-domain information needed to measure the MOCR delay directly in individual ears. Instead, a reset time was chosen that was expected to be sufficient for most ears.

The optimal MOCR reset time has not previously been evaluated in the laboratory for the TEOAE stimulus used here. In general, the issue of reset time for a clinically-focused assay has not been examined in the MOCR literature and is perhaps peculiar to our MOCR assay where repeated trial pairs are measured. In some laboratory studies, researchers have interleaved Q and N recordings but do not mention using either an onset or a reset delay (Goodman, Mertes, Lewis, & Weissbeck, 2013; Lewis, 2019) or the delay was relatively short (e.g., 500ms in Mertes & Goodman, 2016). As already mentioned, other laboratory tests in the literature used time-domain processing where the time course of the efferent activation is directly measurable and lingering efferent activation more easily detectable (e.g., Backus & Guinan, 2006). Many clinical studies take just the one measurement of the OAE with and without the elicitor, with no reset delay applicable, but often these studies then step through a set of parameters in a test session, thereby still potentially exposing subsequent tests to lingering efferent activation (e.g., Wolpert, Heyd, & Wagner, 2014).

If the MOCR reset time can be reduced (or even eliminated), up to 20 seconds per ear could be shaved from the clinical measurement (assuming three trial-pairs). For a laboratory measurement where 15 trial-pairs may be measured in a session, over two minutes may be saved. This saving may be small overall, but with many other demands on test time, every second counts. Furthermore, test quality may also improve because

there would be no long, quiet gaps where the patient or participant is not receiving any auditory input, which can cause restlessness or noise.

1.1 Aims and Prediction

The main aim was to evaluate four reset times and establish their effects on TEOAE and MOCR measurements. Three TEOAE stimulus levels were included to see if higher stimulus levels improved measurability by increasing the signal-to-noise ratio (SNR), and as a secondary issue, whether reset time differentially affected them. The measurability analysis was outside the focus of this report.

Because the MOCR% is calculated as the complex vector difference in the OAE level between the Q and N trials, divided by the amplitude of the OAE level in the Q trial, a change in the Q trial TEOAE could have a complicated relationship with the resulting MOCR%. Thus, a residual effect of the elicitor on the Q trial could show up as (a) decreased TEOAE levels in subsequent Q trials compared to the first Q trial in a trial series (Q1), (b) a change in MOCR% in subsequent trials compared to the MOCR% from the first trial-pair (MOCR1), and (c) an increasing effect for the shorter reset times, compared to 10 seconds.

2 Methods

2.1 Personnel and location

The experiment was conducted at the Sensory Communication Laboratory within the Research Laboratory of Electronics at the Massachusetts Institute of Technology (MIT), under the supervision of Dr. Charlotte Reed. Drs. Lapsley Miller and Marshall from NSMRL provided the experimental design and analysis. Data collection was the responsibility of Dr. Reed's research assistants Zachary Perez and Timothy Villabona, at MIT.

2.2 Informed consent

The MIT internal review board approved the experimental protocol for testing human subjects. All testing was conducted in compliance with regulations and ethical guidelines on experimentation with human subjects. All participants provided informed consent and were paid for their participation in the study.

2.3 Participants

Ten adult participants completed the study. They were required to be 18 to 40 years old and to pass the screening criteria, which are described in Section 3.6.1. Participants were recruited through flyers posted on bulletin boards at MIT and Boston University, through postings at the student employment offices of these two universities, and through word of mouth.

Table 1. Demographics for participants in the study. Only one ear of each participant was tested.

Sex	Participants	Ears	Average Age (years)	Minimum Age (years)	Maximum Age (years)
F	8	8	27	21	40
M	2	2	24	22	25

2.4 Equipment

The Mimosa Acoustics (Champaign, IL) HearID 3.3 system with the contralateral add-on was used for measuring the MOCR and the middle-ear muscle reflex (MEMR), which can confound MOCR measurements. This add-on automated some of the timings previously done manually (Marshall et al., 2014; Mimosa Acoustics, 2007) and also provided a digital broadband noise output. An IBM ThinkPad was used as the PC controller for HearID. An M-Audio (Cumberland, RI) Transit card was used to deliver the digital contralateral elicitor to the earphone.

An ER10C probe with ER2-14A, 14B, or 14C eartips (Etymōtic Research, Elk Grove, IL) was used for the ipsilateral OAE measurement, and an ER2 insert tubephone with ER1-14A eartips (Etymōtic Research, Elk Grove, IL) was used for the contralateral noise elicitor.

A GSI Middle-Ear Analyzer, Model 1733 (Grason-Stadler, Eden Prairie, MN) was used to conduct standard tympanometry.

An Interacoustics Diagnostic audiometer, Model AD229e, with TDH-39P headphones (Interacoustics, Eden Prairie, MN) was used to conduct standard pure-tone audiometry.

2.5 Stimuli

The contralateral elicitor was a 10 kHz broadband low pass noise that was set to a level of 60 dB SPL and digitally output via the M-Audio Transit device for MOCR experiments using an ER2 earphone. Calibration was done in an artificial-ear coupler.

A transient chirp stimulus was chosen for the TEOAE test. Three stimulus levels (47, 50, and 53 dB SPL) were investigated. The level of 47 dB SPL was used in previous studies (Marshall et al., 2014). The higher levels were included to gauge whether they might be more suitable as a stimulus but were not analyzed in-depth here. The TEOAE test was run in nonlinear mode where, for each average, an ensemble of four chirps were output sequentially: three chirps were presented at 47 dB SPL; the fourth chirp was output at a level 9.5 dB higher, but with opposite polarity. The responses to the four chirps were sub-averaged to remove linear artifact (and linear OAEs) before being added to the overall TEOAE response average.

For each Q or N trial, the stimulus ensemble was repeatedly output into the ear canal until 500 averages with acceptable noise levels were collected, or 400 high-noise rejections were made, whichever occurred first. No other stopping rules were used. The noise rejection threshold was initially set in the protocol, but during testing, the tester could adjust it up or down as necessary.

Post hoc, the TEOAE results were reanalyzed to add a microphone equalization and to reanalyze into wider bands, which were found to be optimal in earlier research (Marshall et al., 2014), but had not been analyzed in this way at the time of this experiment. The microphone equalization was able to adjust the response, but the effects of no equalization unavoidably affected the stimulus.

2.6 Procedure

There were four two-hour sessions for each participant: Day 1 was for screening, and Days 2-4 were test days. Participants were required to remain awake during testing because sleep can decrease efferent activity (Froehlich, Collet, Valatx, & Morgon, 1993), but they did not have to attend to the stimuli. They could choose to read quietly. Participants were asked to avoid hazardous noise between Days 1 and 4. The median

number of days between Day 2 and Day 4 was 11 and ranged from 3 to 47 days. Participants underwent a brief rescreening on each test day that included tympanometry, otoscopy, and checking noise-free status. All within-condition trial-pairs were completed in the same session. Different conditions were spread over multiple test days.

2.6.1 Day 1 Screening

Each potential participant underwent the following screening in both ears until one or both ears failed a test. If both ears failed, the participant was not enrolled in the study.

- IRB informed consent was obtained.
- Noise and hearing history were taken orally using standardized questions developed for the OAE-based hearing studies conducted at MIT. The noise history was not used to screen people out but to establish if the participant could remain noise-free during the study. The hearing history was used to ensure there were no medical or other events that may suggest the participant's hearing system was anything other than normal. No potential participants were excluded.
- An otoscopic exam was conducted to ensure clear ear canals.
- Immittance tympanometry with a 226-Hz tone (at a sweep speed of 12.5 daPa/s to minimize hysteresis) was used to screen for peak immittance within 50 daPa of 0 daPa, with grossly normal amplitude, slope, and smoothness of the tympanogram (consistent with normal middle-ear function).
- Participants were required to have normal hearing thresholds, defined as ≤15 dB HL at 0.5, 1, 2, 3, 4, 6, and 8 kHz in the test ear. The contralateral ear was also required to have ≤15 dB HL at 0.5, 1, 2, 3, 4, and 6 kHz, but could have 20 dB HL at 8 kHz. The modified Hughson-Westlake procedure was used with pulsed-tones.
- TEOAE screen (47 dB Shera chirp, nonlinear mode) required a pass in the 1-1.5 and 1.5-2 kHz band. Criteria for a pass was 12 dB SNR for 1-1.5 kHz and **7** dB SNR for 1.5-2 kHz, with an overall level of at least **-7** dB SPL. These numbers were estimated by finding the levels that produced valid MOCR estimates in an earlier experiment.

If both ears passed screening at this point, the ear with the best average audiometric thresholds from 1-3 kHz was chosen as the test ear. Otherwise, the test ear was randomly chosen. Screening then continued with MEMR testing, as follows, in the selected test ear.

- MEMR testing was conducted using Method 1 from Lapsley Miller and Marshall (2014), which considered changes in the ipsilateral DPOAE stimulus level in the presence of a broadband noise contralateral elicitor. Four contralateral elicitor levels were measured: 50, 55, 60, and 65 dB SPL. The test ears had to show no MEMR response greater than 0 dB SPL to contralateral levels below 65 dB SPL, with MEMR test variability also below 0 dB SPL to ensure a response was not masked.
- Similarly, MEMR testing was used with the TEOAE stimulus as the evoking MEMR elicitor to ensure the TEOAE stimulus did not create a MEMR. A second HearID 3.3 system on a separate laptop was used to generate the TEOAE stimuli. Here the TEOAE stimulus was presented to the ipsilateral (test) ear and the MEMR evaluated in the contralateral ear. TEOAE stimulus levels tested were: 44, 47, 50, and 53 dB SPL. Ears had to show no MEMR response greater than 0 dB SPL, with MEMR test variability also below 0 dB SPL to ensure a response was not masked.
- No screening was done for the presence of spontaneous OAEs because previous research had indicated that their presence did not affect TEOAE-based MOCR estimates (Marshall et al., 2014).

- Most participants also received a short MOCR reset series (3-5 trial pairs), using a test similar to previous research with a 47 dB SPL TEOAE stimulus in the test ear, and a 60 dB SPL broadband noise elicitor in the contralateral ear, and a 10s reset time. This series was included to estimate whether the participant had sufficient MOCR strength to warrant further testing. Low MOCR strength would not have allowed the effect of reset time to be seen. Participants were required to have a *detectable* MOCR% (using ROC analysis, as per Marshall et al., 2014), with strength above 20%.

2.6.2 Days 2, 3, and 4

- Days 2-4 started with a quick screening to ensure ear canals were free from cerumen or other debris and that the tympanometric peak pressure was within 50 daPa of 0 daPa The participant was also questioned as to whether they had remained noise-free since the last test session.
- Each test day there were four sessions (at least six MOCR trial-pairs for the four reset times) at one stimulus level, with a short break between each condition, including refitting the probe.
- Reset times were tested in the order: 10, 1.5, 5, 3 seconds.
- Day 2 used a TEOAE stimulus level of 47 dB SPL; Day 3, 50 dB SPL; and Day 4, 53 dB SPL.

2.6.3 Reset time accuracy and other confounds

The control of the elicitor was partly automated. The tester had to accept the current measurement before proceeding with the next. The decision was made during the reset time. Even for the shortest reset times, there was usually time to make this decision before the timer completed. Countdown timers displayed the remaining onset delay and offset delay (which is what the reset time is called in the software package) times. Although the times were automated, because the tester had to manually accept a trial (which also allowed for retesting and recalibration), more than the prescribed reset time may have elapsed. The version of the HearID software used in this experiment did not record the exact start and end time of each measurement (this was added in subsequent releases). Thus the reset times recorded were a *minimum*. If the tester judged too much time had elapsed relative to the timer, the trial was excluded, and a new trial run.

The tester could recalibrate the TEOAE stimulus level if the probe moved or slipped, which potentially could affect the measured TEOAE if the probe was refitted at a different depth.

3 Results

Each participant contributed 5 to 7 trial-pairs of valid data per condition. Four approaches were used to assess the effect of reset time on the TEOAE level and MOCR strength.

3.1 Prediction interval for Q1 based on Qrest for TEOAE level

In a series of QNQNQN… trials, only the first Q trial (Q1) cannot be affected by contralateral efferent activity. This is because there is no preceding N trial, with a broadband contralateral noise that could evoke efferent activity. Subsequent Q trials (Qrest, i.e., the rest of the Q trials) always have a preceding N trial and potentially may be affected by the contralateral elicitor. Rather than look at the derived MOCR strength estimate, which is based on both the Q and N trial TEOAE results, an alternative approach is to consider whether the Q1 TEOAE amplitude was significantly different from Qrest. If there were lingering efferent activity, it would be expected that the Q1 amplitude would be higher than Qrest trials.

To establish if the Q1 trials were different from the Qrest trials, the Qrest trials were used to create a 95% prediction interval. The prediction interval is a confidence interval calculated from a set of measurements and then used to predict the range where a new observation might fall for a given probability. A prediction interval was calculated for each participant and condition, and the corresponding Q1 trial compared to that interval. The proportion of participants with Q1 trials with amplitudes above the prediction interval (hits) were counted separately to Q1 trials with amplitudes below the prediction interval (estimate of false positives). If there were any significant effects, the proportion of participants with high Q1 would be greater than the proportion of participants with low Q1.

These proportions were calculated for all four reset times, all three TEOAE stimulus levels, and three analyzing bands (0.5-2.5 kHz, 1-3 kHz, and the Wideband response). It should be noted that these three bands overlap and are therefore correlated.

Figure 1 shows the percentage of participants with a Q1 TEOAE amplitude higher than the prediction interval (blue), and the percentage with an amplitude lower than the prediction interval, which indicates the false-positive rate (red). These results strongly suggest that there is something different about Q1 for all reset intervals other than 10 seconds. Statistically, there should be an occasional extreme measurement, which is why there is a scattering of red bars. However, in some conditions, 40% of participants (4 of 10) had a Q1 larger than the rest of the Q amplitudes. This result indicates that subsequent Q trials have their amplitudes suppressed by the lingering effects of the contralateral elicitor from the previous trial. The largest effect was for the 53 dB SPL stimulus in the 1-3 kHz band for reset times of 3 and 5 seconds, with 40% of participants showing an increased Q1 amplitude. It was unexpected that the TEOAE amplitudes after the 5-second reset were affected the most and 1.5 seconds the least (ignoring the 10-second baseline condition for now). It would be expected that the shorter the reset time, the more effect the elicitor would have on decreasing the TEOAE amplitude. This result may be due to the small N and also due to the partially-manually timed delays, as described earlier. Both testers were highly-trained and experienced with the software, so they were fast and accurate, but even so, they may have taken slightly longer to start the next trial sometimes. The effect may be that 1.5 and 3 seconds may not be so different in actual duration (which was not possible to measure in this version of the software).

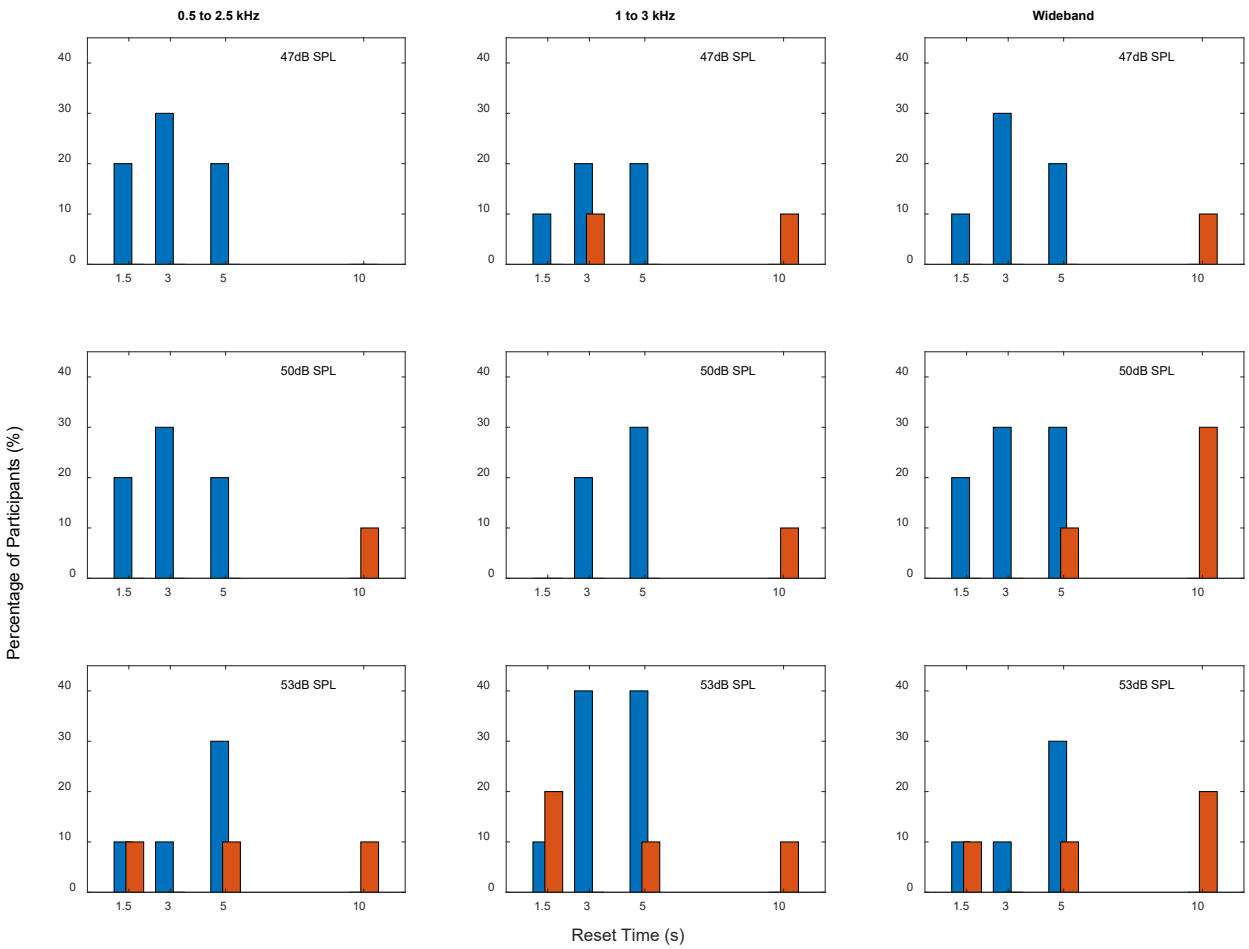

Figure 1. Effect of the MOCR reset time on TEOAE amplitudes for the ten participants (10 ears). Shown is the percentage of participants with TEOAE levels in Q1 outside each participant's prediction interval calculated from the subsequent Q trials (Qrest), separately for each analysis band (column) and stimulus level (row). Blue indicates the percentage of participants with Q1 higher than the prediction interval and red the percentage of participants with Q1 lower than the prediction interval.

This finding was assessed further by collapsing the 1.5, 3, and 5-second conditions across all bandwidths and stimulus levels. Q1 was elevated for 21% trial-pairs and diminished in only 3% of trial pairs (but do note the three analyzing bandwidths are based on the same measurements and are thus highly correlated). Considering the 10s condition collapsed across all other conditions, 0% showed an elevated Q1, and 12% were diminished. Taking 12% as the worst-case false positive rate, the finding that 0% of the 10s condition and 21% of shorter reset times were elevated indicates that shorter reset times affected TEOAE amplitudes.

To review if the effect was specific to some participants, we grouped all conditions and levels and counted the number of cases where the first TEOAE level (Q1) was bigger or smaller than Qrest (Table 2). Two participants contributed most of the false positives but had a correspondingly equal number of hits (Participants 128 and 150), which suggests they had higher variability in general. Three participants consistently showed a higher initial TEOAE level (Participants 134, 146, and 171). The remaining participants showed only a few points with a lower or higher value for the first trial, which could be due to chance.

Table 2. Counts for higher and lower Q1 values for individual participants, grouped across all conditions and levels.

Participant	Total Conditions and Levels included	Count where Q1 was higher than Qrest	Count where Q1 was lower than Qrest
123	36	1	2
128	36	4	6
134	36	19	0
135	36	3	1
144	36	0	1
146	36	11	0
149	36	0	0
150	36	8	9
168	36	3	1
171	36	8	0

Individual cases for smaller Q1 (red) were also investigated to see why the first trial was lower than subsequent trials. A couple of cases were associated with a recalibration between Q1 and Q2, which may have been enough to change the TEOAE level downwards slightly. This finding suggests there may also be some cases included in the larger (blue) category where a recalibration sent the TEOAE level upwards. There was nothing to suggest a systematic effect of recalibration for the different reset times.

3.2 Prediction interval for MOCR1 based on MOCRrest

It appears that there is a difference between the TEOAE amplitude on the Q1 trial compared to subsequent trials (Qrest) for shorter reset times, with the Q1 amplitude tending to be higher than predicted in some ears. Could this also cause the MOCR measured in the first trial (MOCR1) to differ from the MOCR measured in the rest of the trial series (MOCRrest)? If so, is the difference in MOCR strength statistically or clinically significant?

Keep in mind that the TEOAE level from the Q trial is used twice when calculating the normalized MOCR% (calculated as 100(Q-N)/abs(Q)). The impact of Q1 is further complicated by the fact that the Q-N calculation is a complex vector difference. Because Q is also used as a weighting factor (i.e., as the denominator to create the percentage), the effect on the MOCR measure is not linear as a function of Q (assuming N stays the same), although it is a monotonically increasing function for when N<Q. In other words, if subsequent TEOAE levels (Qrest) are smaller than TEOAE levels in Q1, the subsequent MOCR (MOCRrest) will also be smaller than for TrialPair1 (MOCR1). Moreover, the effects of variability are also much higher because rather than one number (OAE amplitude), two numbers are combined, each with their own error, and their respective variances are additive.

Using the same technique as described above for calculating TEOAE prediction intervals, MOCR% prediction intervals were calculated and compared to MOCR1. There was no systematic pattern seen for MOCR% (Figure 2), nor did the results relate to those shown in Figure 1 in any discernible way.

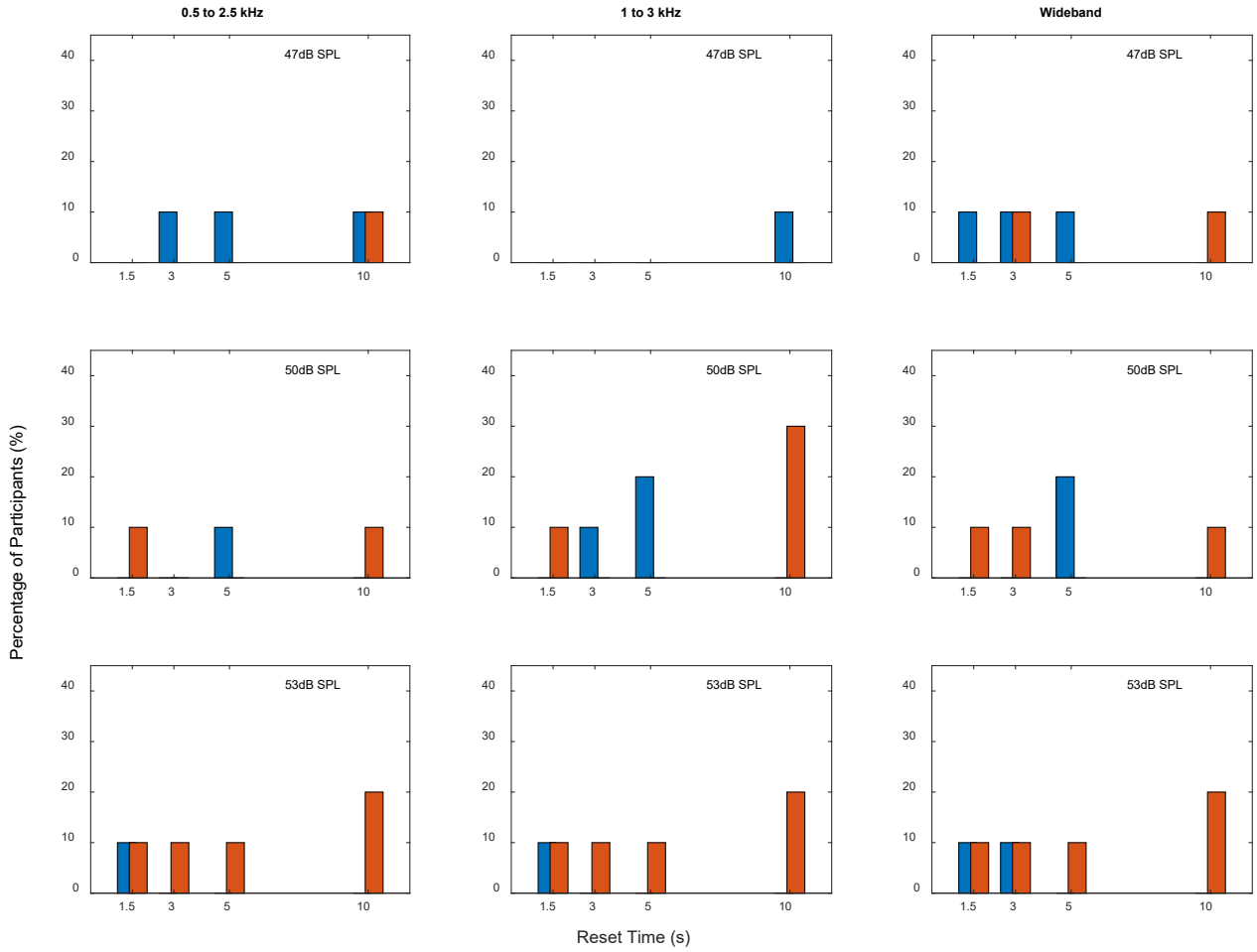

Figure 2. Effect of the MOCR reset time on normalized MOCR strength estimates for the 10 participants (10 ears). Shown is the percentage of participants with MOCR1 levels from the first trial pair outside the participant's prediction interval from the subsequent trials (MOCRrest), separately) for each analysis band (column) and stimulus level (row). Blue indicates the percentage of participants where the first MOCR in the series (MOCR1) was greater than subsequent MOCR (MOCRrest); red where MOCR1 was less than subsequent MOCRrest.

3.3 ANOVA

MOCR strength (MOCR%) is predicted to be different for the shorter reset times, compared to the baseline 10-second reset time.

A repeated-measures ANOVA was conducted for the three stimulus levels (LEV) and the four reset times (RESET). The dependent variable was MOCR% based on the TEOAEs from the 1 to 3 kHz band. With only 10 ears, this ANOVA should be considered suggestive only. All conditions met normality assumptions using the Shapiro-Wilk test. Because all three analysis bands overlap and are therefore correlated (0.5 to 2 kHz, 1 to 3 kHz, and the wideband response which is approximately 0.5 to 6 kHz), only one was selected to be used in the ANOVA. The 1 to 3 kHz band was selected arbitrarily.

There was a main effect for the TEOAE stimulus level, with the lower level producing a higher MOCR% as expected ($F_{2,18}$=9.1, p<0.05). There was also a significant main effect for reset time ($F_{3,27}$=5.4, p<0.05). There were no significant interactions (Figure 3). A Bonferroni post hoc test on all six comparisons among reset times (across all levels) showed one significant difference between 3 and 5s of 40.4 and 43.3%, using a family-wise significance level of p<0.05/6=0.0083. The reset time main effect is plotted in Figure 4.

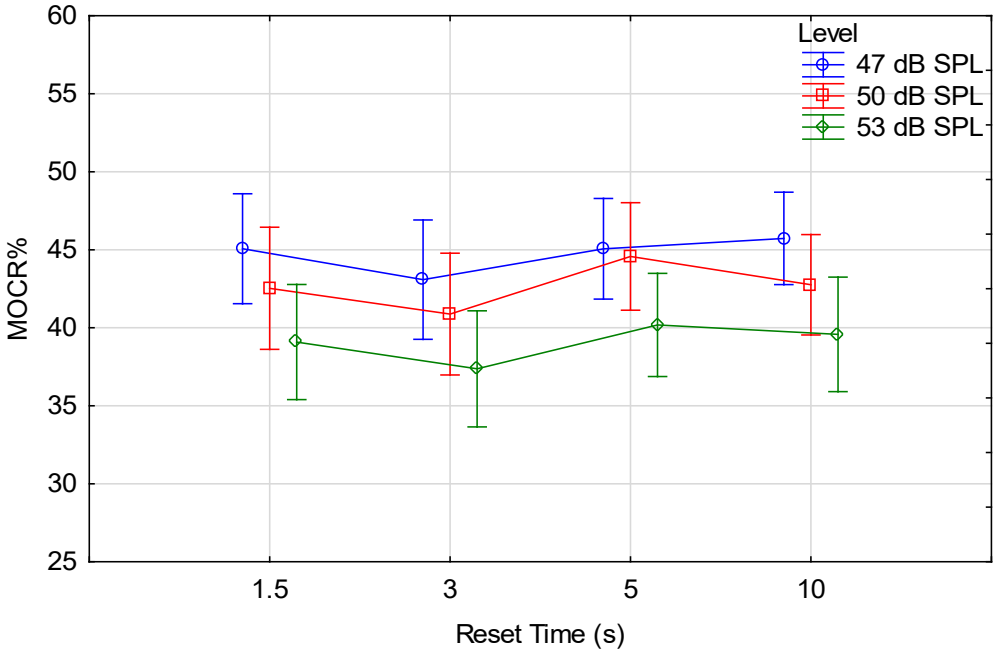

Figure 3. ANOVA interaction plot of average MOCR% by reset time for each stimulus level. Error bars represent the standard errors.

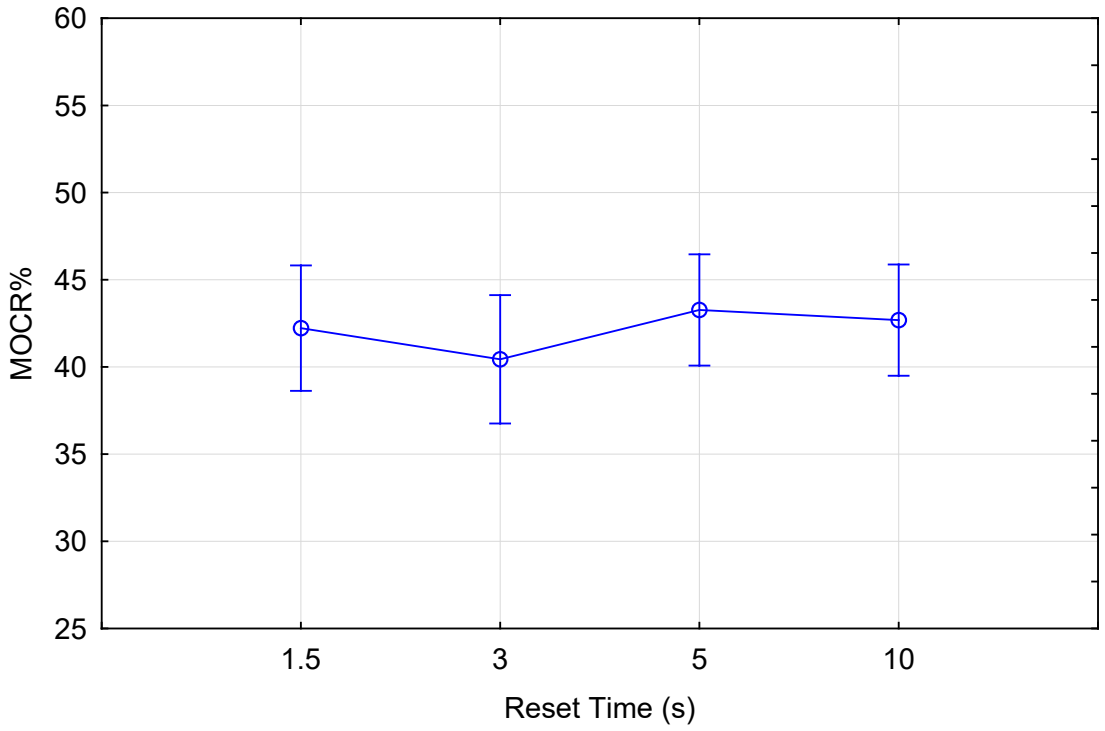

Figure 4. The main effect for Reset Time showed a significantly lower MOCR% for 3 seconds compared to 5 seconds in *post hoc* testing, but no other comparisons among reset time were significant. Error bars represent the standard errors.

3.4 The magnitude of the MOCR1 to MOCRrest difference

The average-MOCRrest was subtracted from MOCR1, and the difference plotted as box-and-whisker plots to gauge the size of the effect. Across all conditions, the average difference was -1.4 percentage points (pp; SD 3.8pp) but with several outliers. The maximum absolute difference was 16.8pp. There did not appear to be a systematic pattern by reset time.

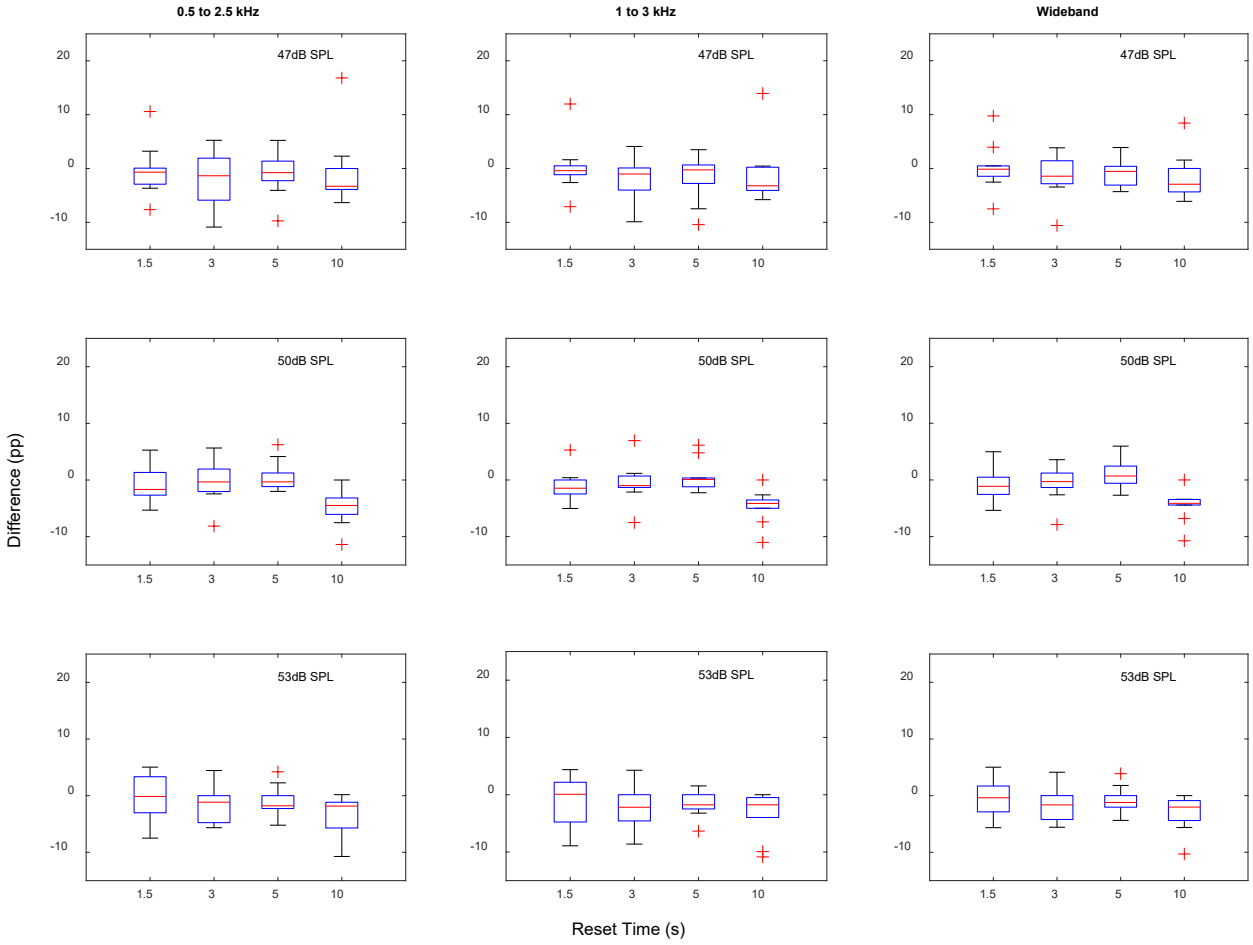

Figure 5. The size of the difference in percentage points between the first MOCR estimate and the subsequent MOCR estimates (averaged) for each analysis band (column) and stimulus level (row). These boxplots show the median (red line), interquartile range (IQR, blue box); the whiskers show the largest or smallest data value within 1.5xIQR, and the red crosses show individual outliers.

4 Discussion

The results of this study indicated we were right to be cautious about our original choice for the reset time in our assay of the medial-olivocochlear reflex. Reset times shorter than 10 seconds show lingering efferent effects interfering with subsequent trials in some ears. However, the findings in this pilot study were not clear-cut. The analyses were approached both by looking for individual and group effects, each giving a unique view.

Prediction intervals for each ear and condition showed if the first Q trial, unaffected by efferent activity, differed from subsequent Q trials. If there were lingering efferent activity, the Q1 TEOAE amplitude would be higher than the prediction interval. The 10 second reset time never produced diminished amplitudes. All other reset times across all stimulus levels showed that 10-40% of participants had diminished TEOAE amplitudes relative to the corresponding Q1 amplitudes (see Figure 1). When using the rate of diminished Q1 amplitudes as a false-positive rate, the rate of elevated amplitudes was still much higher, indicating that the shorter reset times affected the TEOAE amplitude. A closer look at the individual results found that the

TEOAE levels for 30% of ears were consistently affected by the shorter reset durations, but the rest showed no or inconsistent effects.

The prediction-interval analysis for MOCR% provided no systematic pattern for reset times, nor did it reflect the TEOAE amplitude results. This may be due to the complicated way Q propagates through the MOCR% calculation, or it may be primarily false positive results.

An alternative way to look at the effect of reset time on MOCR% was through a repeated-measures ANOVA, though with only 10 ears, care should be taken to not over-interpret the results. The post hoc analysis of the main effect on MOCR% for reset time showed the 3 and 5 second reset times differed significantly from one another but not from 1.5 and 10 seconds. This result is difficult to interpret because the expectation was that any effect on MOCR% should be systematically greater with decreasing duration; thus, it was more likely due to the small N or to the semi-manual timing of the reset delay.

As a minor aim, this study also assessed three TEOAE stimulus levels. In earlier work (Marshall et al., 2014), only the 47 dB SPL condition was used. We were interested in using higher levels to improve signal-to-noise ratio, but were concerned there might be an interaction between level and reset time. The main effect of the TEOAE stimulus level showed that as the level increased, MOCR% decreased. This result was expected for two reasons. In part, the higher stimulus level gave a higher amplitude OAE, which was then a divisor in the MOCR% calculation. It also may have to do with the compressive TEOAE I/O function (TEOAE level as a function of stimulus level). The higher the stimulus level, the smaller the effect of the elicitor on decreasing the TEOAE level. There was no interaction with reset time.

Finally, boxplots were used to assess the distribution of the differences between Q1 and Qrest for MOCR%, with no systematic pattern unfolding. The pattern of results for the boxplots was similar to the bar graphs shown in Figure 2: as expected, the boxplots showing positively-skewed difference distributions could be matched with those conditions where MOCRrest was larger than MOCR 1 (blue bars), and the negatively-skewed boxplots could be matched to the conditions where MOCR1 was larger than MOCRrest (red bars).

Several limitations affected what could be concluded from the results. As mentioned above, only 10 participants completed the study, and only 5-7 trial-pairs were taken for each condition. Timings were approximate in this semi-manually-timed study, and the actual timings were not recordable in the version of HearID used. The actual durations for the 1.5, 3, and 5-second resets may not have been as distinct as desired, but all three were likely faster than the 10-second baseline. Thirdly, stimulus recalibrations may have affected TEOAE levels, though the rate of recalibration is somewhat random and is expected to be independent of the experimental variables.

This paper outlines methods to evaluate the effects of reset time but does not fully answer the question of the impact of reset time on TEOAE-based assays. Future investigations should use more sophisticated equipment to evaluate the effect of reset time. Both the Mimosa Acoustics HearID and OtoStat systems are now automatically timed, and all timings are recorded to file. Both systems have accurate inbuilt microphone equalizations. OtoStat has more accurate in-the-ear calibrations based on forward-pressure level calibration for both the ipsilateral TEOAE test and the contralateral elicitor. Newer versions of the MOCR assay are also faster to run and have a lower effective stimulus level. Whether these improvements affect the minimum reset time remains to be seen. It is recommended that the reset time is reevaluated using

these improvements as the 10-second limitation significantly slows down the test when measuring multiple trial-pairs.

In summary, subtle trends indicative of reset time effects were observable by evaluating the TEOAE level of Q1 versus the subsequent TEOAE levels in the trial series, but there was no systematic finding when evaluating MOCR%. Until a more extensive investigation is conducted with a larger sample and tighter control over the reset timings, we recommend that 10 seconds should continue to be used as a conservative reset time when running a MOCR assay, based on the protocols developed in Marshall et al. (2014). The analysis methods developed here might prove helpful in analyzing and interpreting the results in future studies.

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6 APPENDIX A: List of Acronyms

DPOAE: distortion-product otoacoustic emission – a sound evoked in the inner ear by two tonal stimuli and measured in the ear canal with a sensitive microphone.

I/O function: input/output function

Q: a “quiet” trial where no contralateral elicitor is used

QN: a single Q and N trial-pair, from which a MOCR estimate may be derived by comparing the vector difference in the OAE level.

MEMR: middle-ear muscle reflex

MIT: Massachusetts Institute of Technology.

MOCR: medial olivocochlear reflex

MOCR%: MOCR derived strength statistic, which is expressed as a percentage. It is calculated by taking the vector difference in the TEOAE level between the Q and N trial and then normalized by dividing by the magnitude of the Q trial TEOAE level. Normalizing means the MOCR strength is independent of the OAE level.

MOCR1: the MOCR% for the first QN trial pair

MOCRrest: the MOCR% for trials after the first QN trial pair

N: a “noise” trial where the broadband noise elicitor is used to suppress the MOCR.

NSMRL: Naval Submarine Medical Research Laboratory.

OAE: otoacoustic emission. A general term that does not describe the specific evoking stimulus.

pp: percentage points

SNR: signal-to-noise ratio (dB)

TEOAE: transient-evoked otoacoustic emission – a sound evoked in the inner ear by a transient stimulus and measured in the ear canal with a sensitive microphone. It can be suppressed by eliciting the MOCR with a contralateral stimulus via the auditory efferent system.

Time course: Q1 – (2-second onset) – elicitor – N1 – (X-second reset) – Q2 … where X in this study was an independent variable with values: 1.5, 3, 5, and 10 seconds.