Naval Submarine Medical Research Laboratory

NSMRL/50904/TR--2021-1345 June 11, 2021

Otoacoustic emission (OAE)-based assays of the medial-olivocochlear reflex (MOCR): ensuring the OAE stimulus does not cause a confounding MOCR

by:
Judi A Lapsley Miller
Naval Submarine Medical Research Laboratory & Mimosa Acoustics, Inc.

Lynne Marshall
Naval Submarine Medical Research Laboratory & University of Connecticut

Charlotte M. Reed
Zachary D. Perez
Research Laboratory of Electronics, Massachusetts Institute of Technology

Approved and Released by:
K. K. Shobe, CAPT, MSC, USN
Commanding Officer
NAVSUBMEDRSCHLAB

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
11-06-2021	Technical Report	Sep 2008-May 2009; Dec 2019; May 2020

4. TITLE AND SUBTITLE

Otoacoustic emission (OAE)-based assays of the medial-olivocochlear reflex (MOCR): ensuring the OAE stimulus does not cause a confounding MOCR

5a. CONTRACT NUMBER

5b. GRANT NUMBER

N0001412WX20904

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Judi A Lapsley Miller
Lynne Marshall
Charlotte M Reed
Zachary Perez

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

50904

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Submarine Medical Research Laboratory
Box 900
Groton, CT 06349-5900

8. PERFORMING ORGANIZATION REPORT NUMBER

NSMRL/50904/TR--2021-1345

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
One Liberty Center
875 N. Randolph Street, Suite 1425
Arlington, VA 22203-1995

10. SPONSOR/MONITOR'S ACRONYM(S)

ONR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for Public Release, Distribution is Unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In the transient-evoked otoacoustic emission (TEOAE)-based medial-olivocochlear reflex (MOCR) assay developed by Marshall et al. (2014), a higher TEOAE stimulus level could improve TEOAE signal-to-noise ratio and MOCR measurability. However, a higher level may evoke a confounding ipsilateral MOCR in addition to the contralateral MOCR from the broadband noise elicitor. This study was designed to establish if the TEOAE chirp stimulus could evoke a MOCR by using it as the contralateral MOCR elicitor in place of the broadband noise. A stimulus-frequency otoacoustic emission (SFOAE)-based MOCR assay was used because tonal SFOAE stimuli do not elicit a confounding MOCR at the levels used.

15. SUBJECT TERMS

medial-olivocochlear reflex, transient evoked otoacoustic emission, stimulus-frequency otoacoustic emission

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			NAVSUBMEDRSCHLAB Commanding Officer
U	U	U	SAR	30	19b. TELEPHONE NUMBER *(Include area code)* 860-694-3263

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

[THIS PAGE INTENTIONALLY LEFT BLANK]

Otoacoustic emission (OAE)-based assays of the medial-olivocochlear reflex (MOCR): ensuring the OAE stimulus does not cause a confounding MOCR

Judi A Lapsley Miller
Naval Submarine Medical Research Laboratory & Mimosa Acoustics, Inc.

Lynne Marshall
Naval Submarine Medical Research Laboratory & University of Connecticut

Charlotte M. Reed
Zachary D. Perez
Research Laboratory of Electronics, Massachusetts Institute of Technology

Naval Submarine Medical Research Laboratory

Approved and Released by:

SHOBE.KATHARINE.KRAUSE.1242518280

Digitally signed by SHOBE.KATHARINE.KRAUSE.1242518280
Date: 2021.07.27 10:44:52 -04'00'

K. K. Shobe, CAPT, MSC, USN
Commanding Officer
Naval Submarine Medical Research Laboratory
Submarine Base New London Box 900
Groton, CT 06349-5900

Administrative Information:

This work was conducted/funded by Work Unit #50904. The study protocol MIT 0603001665 was approved by the Committee on the Use of Humans as Experimental Subjects (COUCHES) established to act as the Institutional Review Board (IRB) for the Massachusetts Institute of Technology (MIT) in compliance with all applicable Federal regulations governing the protection of human subjects. The views expressed in this report are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government. This work was prepared by employees of the U.S. Government as part of their official duties. Title 17 U.S.C. §105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

[THIS PAGE INTENTIONALLY LEFT BLANK]

ABSTRACT

The medial-olivocochlear reflex (MOCR) is an auditory efferent reflex that might predict risk for noise-induced hearing loss in humans. It also has other audiological applications. To translate a laboratory assay to field trials and clinical use in hearing-conservation programs, various confounds and test inefficiencies must be addressed. This report considers at what level the stimulus itself evokes an MOCR. In the transient-evoked otoacoustic emission (TEOAE)-based MOCR assay developed by Marshall *et al.* (2014), a higher TEOAE stimulus level could improve TEOAE signal-to-noise ratio and thus the number of ears the MOCR can be measured in. However, a higher level may evoke a confounding ipsilateral MOCR in addition to the contralateral MOCR from the broadband noise MOCR elicitor. This study was designed to establish if the TEOAE chirp stimulus could evoke a MOCR by using it as the contralateral MOCR elicitor in place of the broadband noise. A stimulus-frequency otoacoustic emission (SFOAE)-based MOCR assay was used because tonal SFOAE stimuli do not elicit a confounding MOCR at the levels used. Fifteen participants were tested in one ear. For each participant, the SFOAE-MOCR responses to six individually-selected frequencies were measured in a series of nine trial-pairs per frequency. Four contralateral TEOAE stimulus sound pressure levels (SPL) were evaluated: 44, 47 (used in Marshall *et al.*, 2014), 50, and 53 dB SPL, presented in nonlinear mode, where every fourth stimulus presentation was 9.5 dB higher and with opposite polarity. The normalized MOCR strength was averaged across frequency and trial-pair for each participant and level. There was no evidence of MOCR activity at the 44 or 47 dB SPL levels. Three of the 15 ears showed a response to the 53 dB SPL elicitor. A repeated-measures ANOVA showed only the 53 dB SPL level produced a significant confounding MOCR reaction. It is recommended that studies using the nonlinear TEOAE mode for MOCR assays use stimulus levels at 50 dB SPL or lower to ensure an ipsilateral MOCR is not also evoked.

ACKNOWLEDGEMENTS

Thanks go to the Noise-Induced Hearing Loss (NIHL) Program at ONR 342 and its program officer Kurt Yankaskas (retired) for their ongoing support for translating MOCR basic research into a clinically viable assay. Thanks to Linton Miller for his assistance with the SFOAE test point selection algorithm. Thanks also to the staff at Mimosa Acoustics for supporting the MOCR development on their HearID platform.

Contents

Figures

Tables

Executive Summary

Issues: The medial-olivocochlear reflex (MOCR) is an auditory efferent reflex that might predict risk for noise-induced hearing loss in humans. It also has other audiological applications. To translate a laboratory assay developed by Marshall et al. (2014) to field trials and clinical use in hearing-conservation programs, various confounds and test inefficiencies must be addressed. This report considers at what level the stimulus itself evokes an MOCR. A higher ipsilateral transient-evoked otoacoustic emission (TEOAE) stimulus level could improve the TEOAE signal-to-noise ratio and thus the number of ears in which the MOCR can be measured.) However, a higher level may evoke a confounding ipsilateral MOCR in addition to the contralateral MOCR from the broadband noise elicitor.

Objective: To establish if the TEOAE stimulus can evoke a MOCR, and at what stimulus level this may occur.

Approach: A stimulus-frequency otoacoustic emission (SFOAE)-based MOCR assay was used to assess whether the TEOAE chirp stimulus, presented contralaterally, could evoke a MOCR. This chirp stimulus replaced the usual contralateral broadband noise elicitor. Four TEOAE stimulus levels were tested: 44, 47 (used in Marshall et al., 2014), 50, and 53 dB SPL. Fifteen participants were tested.

Results: There was no evidence of MOCR activity to the 44 or 47 dB SPL levels. Three of the 15 ears showed a small response to the 53 dB SPL elicitor. A repeated-measures ANOVA indicated only the 53 dB SPL elicitor produced a significant MOCR effect.

1 Introduction

The medial-olivocochlear reflex (MOCR) is of great interest to military audiologists because it has the promise of predicting which ears are more vulnerable to damage from noise exposure (Marshall *et al.*, 2014). The mechanisms as to why this might be the case are not fully understood but there are a range of possibilities (summarized by Marshall and Lapsley Miller, 2014). Although animal studies are promising (e.g., Maison and Liberman, 2000; Luebke and Foster, 2002; Maison *et al.*, 2013; Liberman *et al.*, 2014), there is limited evidence of the reflex's protective abilities in humans (e.g., Shupak *et al.*, 2007; Muller and Janssen, 2008; Muller *et al.*, 2010; Wolpert *et al.*, 2014, as summarized in Marshall et al., 2014). In part this is due to poorly designed assays (summarized by Keppler *et al.*, 2021) along with the obvious ethical restrictions and limitations in studying the effects of damaging noise on humans. Our approach is to refine an assay to ensure it has the necessary statistical properties to detect a range of MOCR values in the population (Marshall *et al.*, 2014) while minimizing potential confounds and maximing test efficiency for use in time-sensitive settings (Lapsley Miller and Marshall, 2014; Lapsley Miller *et al.*, in preparation-a; Lapsley Miller *et al.*, in preparation-b; Lapsley Miller *et al.*, in preparation-c). Once these refinements have been made, the assay will be ready for use in the expensive longitudinal field trials that are necessary to establish clinical efficacy.

The TEOAE-based MOCR assay, developed by the Naval Submarine Medical Research Laboratory (Marshall *et al.*, 2014), requires further development before it is clinically viable. Through a series of experiments and equipment developments, we systematically investigated potential improvements.[1] One

[1] To put the current study into context, data collection was conducted in 2008-2009, following on from the experiment reported in Marshall et al. (2014), which was conducted in 2007-2008.

factor precluding its widespread use in the clinic and hearing-conservation programs is that the assay is not measurable in every normal-hearing ear. Here we report on whether it is possible to improve measurability by increasing the TEOAE stimulus level without this stimulus also evoking a confounding MOCR.

The MOCR assay involves measuring an OAE in the ipsilateral (test) ear with and without a broadband noise contralateral elicitor. Trials without the elicitor are called "Q" or "quiet" trials and should not elicit the MOCR. Trials with the elicitor are called "N" or "noise" trials, and are designed to elicit the MOCR. The vector difference in the OAE response level with and without the elicitor defines the MOCR strength, and this difference is typically normalized by the OAE response level measured without the elicitor. This normalized MOCR (MOCR%) is reported as a percentage (Marshall *et al.*, 2014). For a MOCR to be measurable (i.e., distinct from inherent test variability), the underlying OAE test must have sufficient signal-to-noise ratio (SNR) (e.g., Goodman *et al.*, 2013; Lewis, 2018; 2019; Lapsley Miller *et al.*, in preparation-b). SNR can be improved by longer averaging times, but the test must also be fast to administer in clinical settings. An alternative way to achieve an increase in SNR is to increase the TEOAE stimulus level. However, an increase in stimulus level also increases the chances that the TEOAE stimulus itself might act as a MOCR elicitor, confounding the test results (Guinan *et al.*, 2003). Only the contralateral broadband noise (BBN) should be eliciting the reflex in this assay. It is considered a confound because it could elicit the MOCR during a Q trial, thereby decreasing the measured MOCR strength.

To assess the sound pressure level at which the TEOAE chirp stimulus is capable of eliciting a confounding MOCR, we switched from using it as the OAE test stimulus and instead used it in place of the contralateral broadband noise elicitor (see Table 1 to clarify which ear is presented which stimulus). This approach makes the reasonable assumption that a stimulus capable of evoking an ipsilateral reflex is also capable of evoking a contralateral reflex.

Table 1. Overview of the previous and current studies, clarifying which stimulus is presented to which ear.

Assay	Ipsilateral/Test ear	Contralateral	Note
TEOAE-based MOCR assay (Marshall et al. 2014)	Nonlinear TEOAE: chirp stimuli at 47 dB SPL	60 dB SPL BBN	Concern that the TEOAE stimulus might also elicit an ipsilateral MOCR if the stimulus level is increased
SFOAE-based MOCR assay (Marshall et al. 2014)	SFOAE: tonal stimuli at 40/60 dB SPL	60 dB SPL BBN	Tones at these levels do not produce ipsilateral MOCR
Current study	SFOAE: tonal stimuli at 40/60 dB SPL	Non-linear TEOAE, chirp stimuli at 44, 47, 50, and 53 dB SPL	Use TEOAE stimuli as the contralateral elicitor and the SFOAE assay to gauge at what level the TEOAE stimuli can elicit a MOCR

To measure the MOCR strength to the TEOAE stimulus elicitor, we used the SFOAE-based version of the MOCR assay (Marshall *et al.*, 2014). It is well-established that these tonal SFOAE stimuli (40 and 60 dB SPL) do not elicit a MOCR at these stimulus levels (Guinan *et al.*, 2003). Transient stimuli (used here as the contralateral elicitor), in comparison to tones, are more efficient efferent elicitors (Guinan *et al.*, 2003). Unlike the low-level tones, the ability of a transient stimulus to elicit a reflex depends on many parameters

such as click rate, stimulus level, and stimulus shape (e.g., clicks, tone pips, chirps), so it is important to evaluate the specific stimulus used in the assay rather than taking values from the literature where parameters may differ in meaningful ways.

The TEOAE stimulus assessed here was the "Shera chirp" stimulus in the Mimosa Acoustics HearID system, presented in nonlinear mode (Lapsley Miller *et al.*, 2004a). Four TEOAE stimulus levels were tested: 44, 47, 50, and 53 dB SPL RMS in the contralateral ear. The nonlinear mode meant that every fourth stimulus was 9.5 dB higher than the target level but with opposite polarity. This mode was used because it generated less stimulus artifact on an earlier version of the Mimosa equipment (Lapsley Miller *et al.*, 2004a). The chirp stimulus has a longer duration than the equivalent click stimulus and a lower peak-to-rms level, which theoretically should decrease its efficiency as an efferent elicitor compared to a click stimulus.

If the TEOAE stimulus was acting as a MOCR elicitor in this study, we hypothesized that the MOCR strength would be higher than the inherent test variability, and it would increase as a function of increasing stimulus level. We aimed to evaluate if this pattern was seen in individual ears and the average group results, and, if it was seen, to assess which stimulus levels could be used without eliciting a counfounding MOCR and which should not be used in the TEOAE-based MOCR assay.

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 research assistant Zachary Perez.

2.2 Participants

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.

Rigid screening criteria meant approximately half the potential participants did not pass the screening. Nineteen participants were enrolled in the study, and 15 completed data collection. They were recruited and screened similarly to Marshall *et al.* (2014) other than a difference in the requirements for the presence and spread of SFOAEs (see Section 2.5.1 and Appendix B). Each subject contributed data from only one ear, even if both ears passed the screening. The following results presented are for the 15 participants who completed the study. Nine left ears and six right ears were tested. Ten participants were female, aged 18-30 years (average 22 years), and five participants were male, aged 19-32 years (average 25 years).

2.3 Equipment

The Mimosa Acoustics (Champaign, IL) SFOAE-SG v3.0.16 system was used for the SFOAE-based MOCR data collection. The Mimosa Acoustics HearID v3.3 system was used to generate the contralateral TEOAE stimuli. An IBM T43 ThinkPad (Armonk, NY) 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.

Tympanometry was conducted with a GSI Middle-Ear Analyzer, Model: 1733 (Grason-Stadler, Eden Prairie, MN).

Audiometry was conducted with an Interacoustics Diagnostic Audiometer, Model: AD229e, with TDH-39P headphones (Eden Prairie, MN).

2.4 Stimuli

The SFOAE stimulus levels were 40/60 dB SPL for the probe and suppressor, with the suppressor frequency set 47 Hz higher than the probe.

The contralateral TEOAE stimulus was a transient chirp ensemble repeatedly output for the duration of the SFOAE test (during N trials). The stimuli were generated using a separate HearID system, which was manually turned on and off by the tester. The bandwidth was 1-5 kHz, the effective duration was 6.5 ms, and the absolute duration was 10 ms. There was a 20 ms inter-stimulus-interval. With overhead, a chirp was therefore presented every 32.5 ms. The stimulus was calibrated in-the-ear using spectrum calibration (but without a microphone equalization). Four TEOAE stimulus levels were tested: 44, 47, 50, and 53 dB SPL. Stimulus output was via the ER10C probe.

The nonlinear TEOAE mode was used, where four stimuli were output consecutively in an ensemble. Three of the four stimuli were at the target stimulus level, and the other had the opposite polarity and was three times as strong (9.5 dB higher). By sub-averaging the ensemble, linear artifacts are removed. The thing of note here is that the one higher stimulus in the ensemble is more likely to elicit MOCR activity.

2.5 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 MOCR testing because sleep can decrease efferent activity (Froehlich *et al.*, 1993), but they did not have to attend to the sounds presented in their ear canals. They could choose to read quietly. Participants were asked to avoid hazardous noise between Days 1 and 4. The test days spanned across two to 25 days, depending on participant availability. Some participants completed two sessions in one day with a break between sessions.

2.5.1 Day 1 Screening

The tester stepped both ears of each potential participant through the screening procedure until one or both ears did not meet a criterion. If one ear did not meet a criterion, screening continued for the other ear until it either did or did not pass the screening criteria. If both ears passed audiometric screening criteria for the test ear, the ear with the lowest average hearing thresholds from 1-3 kHz was chosen as the provisional test ear; otherwise, the provisional test ear was chosen randomly. The final test ear was selected, and individual SFOAE test frequencies selected overnight, after more extensive data analysis by the offsite scientist, who ensured the test ear showed no middle-ear muscle reflex (MEMR) to the stimulus levels used in the experiment.

Screening proceeded in the following order:

- IRB informed consent 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, and grossly normal amplitude, slope, and smoothness of the tympanogram, consistent with normal middle-ear function.

- Participants were required to have hearing thresholds ≤15 dB HL at 0.5, 1, 2, 3, 4, 6, and 8 kHz in the test ear. The contralateral ear could have 20 dB HL at 8 kHz. The modified Hughson-Westlake procedure was used with pulsed-tones (ANSI/ASA, 2009).

- 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 from an independent dataset by evaluating which TEOAE levels produced valid MOCR results.

- MEMR testing was conducted using Method 1 from Lapsley Miller and Marshall (2014), which considered changes in ipsilateral distortion-product otoacoustic emission (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 done 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 – the same levels used for evaluating whether the TEOAE stimulus elicited a MOCR in the current study. 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.

- Participants had to have six SFOAE frequencies with sufficient amplitude that also were not close to any spontaneous otoacoustic emission (SOAE) activity and were spaced at least 23 Hz apart from each other. These SFOAE frequencies were determined by measuring SFOAEs in the potential test ears at 59 frequencies between 1-2 kHz, then using a heuristic algorithm to determine the optimal test frequencies individually for each ear. If a solution of six frequencies was not found, the participant was not enrolled in the study. Six frequencies were tested to

reduce potential MOCR strength variation across nearby individual frequencies, which had been seen with a different, but related, SFOAE-based assay (Backus and Guinan, 2007). See Table 2 for the frequencies selected for each ear. See Appendix B for the method and examples.

Table 2. The six SFOAE frequencies individually selected for each participant based on SFOAE amplitude and proximity to SOAEs.

Participant	Ear	Freq1	Freq2	Freq3	Freq4	Freq5	Freq6
110	L	1125	1160	1207	1230	1266	1289
109	R	1711	1734	1770	1793	1816	1840
112	R	1512	1535	1875	1898	1922	1945
108	L	1734	1758	1840	1863	1957	1980
115	R	1020	1043	1125	1148	1184	1207
106	L	1008	1031	1055	1078	1125	1148
117	L	1863	1910	1934	1957	1980	2004
118	L	1289	1453	1477	1500	1523	1547
119	L	1371	1395	1746	1770	1793	1816
123	R	1078	1219	1242	1266	1313	1430
124	L	1875	1898	1922	1945	1969	1992
128	L	1055	1078	1102	1125	1207	1230
135	L	1031	1055	1137	1160	1336	1359
134	R	1734	1758	1781	1805	1828	1852
140	R	996	1020	1043	1066	1102	1125

2.5.2 Test Days 2, 3, and 4

The test days 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.

On each test day, eight conditions were tested: two of the six SFOAE frequencies at each of the four TEOAE contralateral elicitor levels. TEOAE elicitor levels were tested in the order: 44, 47, 50, and 53 dB SPL. For each SFOAE frequency, all TEOAE elicitor levels were tested before moving to the next SFOAE frequency. SFOAE frequencies were tested in ascending order over the three days.

SFOAE-MOCR testing used the same method as described in Marshall *et al.* (2014). Each trial-pair consisted of a quiet (Q) trial, which was a SFOAE test at one frequency with no contralateral elicitor, and a noise (N) trial, which was an SFOAE test at one frequency along with the contralateral elicitor. In this experiment, rather than broadband noise, the contralateral elicitor was the TEOAE stimulus under investigation. After the Q trial, the contralateral elicitor was turned on for two seconds before starting the SFOAE test for the N trial. After the SFOAE test finished in the N trial, the elicitor was turned off and 10 seconds allowed to elapse before starting the next Q trial. This ensured that any residual MOCR activity had entirely dissipated (Lapsley Miller *et al.*, in preparation-a). A maximum of 16 low-noise averages were collected in each SFOAE test, and up to 64 rejections allowed before the test stopped. Wideband artifact rejection was used.

For each condition, a series of approximately nine MOCR QN trials-pairs were measured, with a short break between each condition, including refitting the probes. The probes were not to be refitted within a series. If they needed adjusting, the series was repeated or terminated early, depending on how far the tester had gotten and the remaining time in the session.

2.5.3 Data cleaning, definitions, and reduction

The data were screened as for Marshall *et al.* (2014). Both the Q and the N trial SFOAEs contributing to an individual MOCR% estimate had to meet quality control criteria: OAE SNR ≥ 9 dB; OAE noise level < -9 dB SPL; the two SFOAE tones within 3 dB of target levels, and the absolute difference in their levels no more than 3 dB from the target difference.

The MOCR% was calculated for each trial-pair and frequency as usual (Marshall *et al.*, 2014), but then we also calculated an aggregate MOCR% averaged across trial-pair and frequency rather than reporting them as individual test frequencies as previously. This aggregate was used for all the analyses reported here. The goal was to achieve nine trial pairs at six frequencies (54 data points in total) at each stimulus level per subject. For MOCR% based on QN trial-pair combinations, 49 to 69 trial-pairs contributed to each aggregate point (a point is an individual ear's result at one stimulus level across all six frequencies and trial-pairs).

As in Marshall *et al.* (2014), a similar calculation was made to estimate MOCR test variability, with Q and N trials being re-paired into adjacent QQ and NN trials, which provided an estimate of the noise inherent in the measurement. For MOCR variability, 44 to 66 trial-pairs contributed to each point.

3 Results & Discussion

First, results for individual ears were plotted and interpreted, and patterns summarized descriptively. Then a repeated-measures ANOVA was conducted on the group to look for TEOAE stimulus level effects (IV, repeated measure) on the MOCR% (DV), comparing MOCR% to the inherent test variability.

3.1 Individual results

It was expected there would be variability across individuals concerning the TEOAE stimulus level needed to elicit a detectable MOCR, so the first analysis considered individual ears to see if there were any participants showing effects that might not show up when included in the group average.

In Figure 1, the x-axis is the TEOAE stimulus level, the y-axis is the aggregate MOCR%, the blue line is MOCR% for QN trials, and the magenta line is the estimated inherent MOCR variability from the re-paired QQ and NN trials. If there was no MOCR at all, the blue and magenta lines were expected to be flat and overlay one another. If there was a MOCR, it was hypothesized that the higher stimulus levels would show a stronger MOCR effect and that there would be a separation between the MOCR% and the variability (i.e., the blue line would rise above the magenta line).

Without random noise and variation, SFOAE-based MOCR% can take on values between 0 and 100%. Because it is based on a vector difference, MOCR% has a true minimum at 0%, but due to random fluctuations in the SFOAE measurement, there is an effective noise floor of around 10% (Marshall *et al.*, 2014). This noise floor is also seen in the MOCR variability estimate, which can vary across individuals (e.g., compare 112R with 110L). In many cases shown in Figure 1, MOCR% to the TEOAE stimulus elicitor and the MOCR variability was only around 10%, indicating that there was little-to-no MOCR activity.

It was not known before this study if these participants had a measurable MOCR to the regular 60 dB SPL broadband noise. If any of them had low MOCR strength, then they would not be able to show the potentially more subtle effects of the contralateral TEOAE elicitor. However, eight of 15 participants also participated in a subsequent experiment that used a TEOAE-based assay with broadband noise to elicit the MOCR (Lapsley Miller *et al.*, in preparation-a). They all had normal to high MOCR strength. The wideband MOCR% to the broadband noise (BBN) elicitor was used for this assessment based on norms from Marshall et al. (2014).

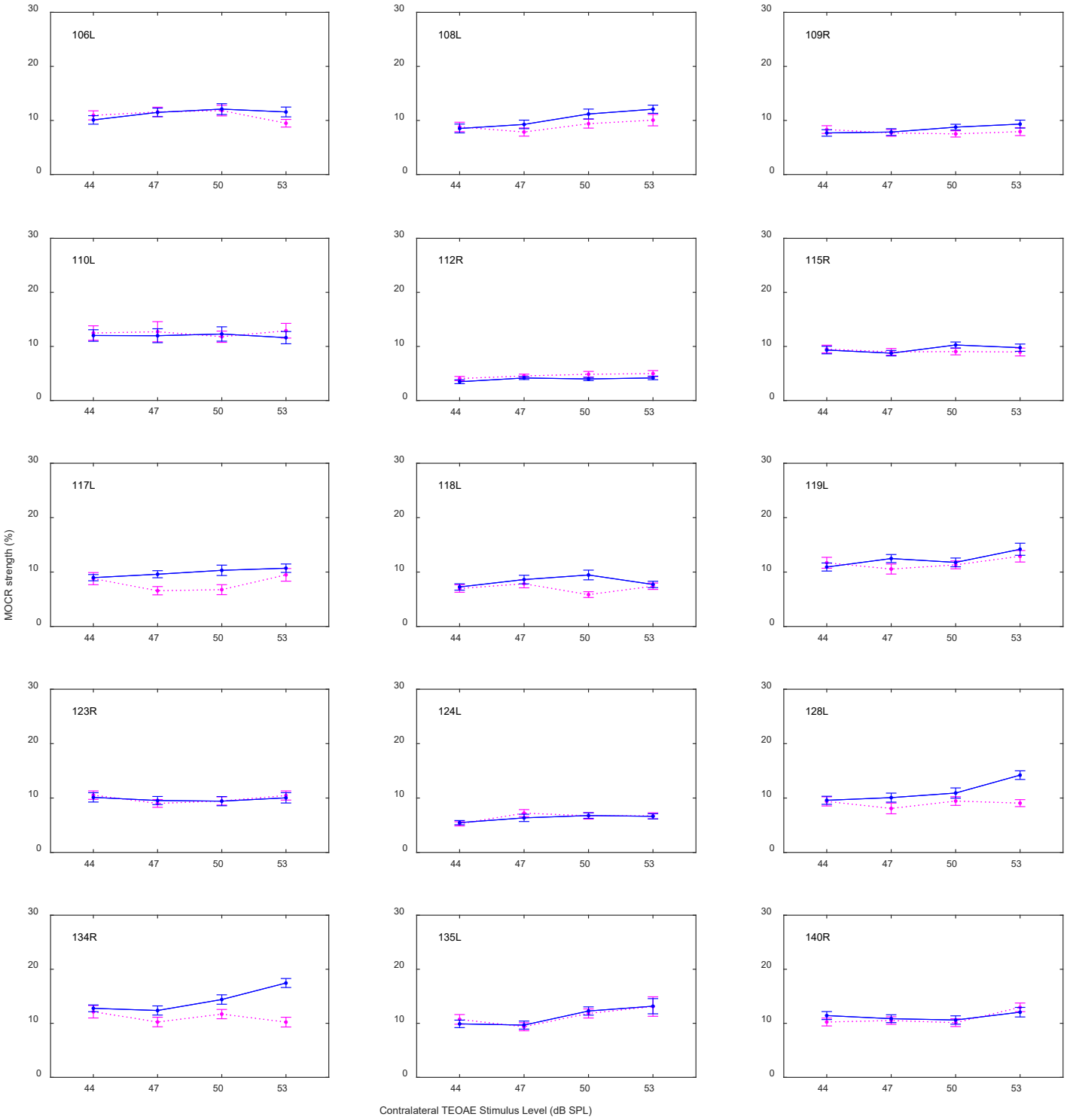

Figure 1. Average MOCR strength estimates (averaged over trial-pairs and frequency) for each participant at each contralateral TEOAE stimulus level (blue line) compared with an estimate of the MOCR variability (dotted magenta line). Error bars represent the standard error.

Visible inspection of each participant's graphs separately may be summarized descriptively as follows:

- Participant 106L: the difference at 53 dB SPL is partly due to the variability dropping rather than the MOCR% rising. The very slight systematic rise in MOCR% with level may be random.

- Participant 108L: there is a small but systematic rise in MOCR% but also in the MOCR variability too. There may be a small effect at 50 and 53. The difference between 44 and 53 dB SPL is 3.6pp, but the difference between MOCR% and MOCR variability at 53 dB SPL is only 2pp.

- Participant 109R: there is a suggestion of a slight MOCR effect at 50 and 53 dB SPL, but the evidence is not compelling.

- Participant 110L showed higher MOCR variability, but no evidence of any MOCR activity. With high variability, this ear is unlikely to reveal any effect of the stimulus level.

- Participant 112R showed very low MOCR variability and no evidence of any MOCR activity.

- Participant 115R showed average MOCR variability, but little to no evidence of any MOCR activity.

- Participant 117L: the differences at 47 and 50 dB SPL are not compelling because there is little difference at 53 dB SPL. The MOCR% was mostly flat over level, with perhaps a slight increase with level. The differences seen are more due to a dip in MOCR variability.

- Participant 118L showed a difference at 50 dB SPL that was not supported by a difference at 53 dB SPL. There was no systematic increase with level.

- Participant 119L: there was a systematic increase in MOCR% but also an increase in MOCR variability, so we cannot ascribe the pattern to MOCR activity.

- Participant 123R showed average MOCR variability, but no evidence of any MOCR activity. The TEOAE-MOCR was normal.

- Participant 124L showed very low MOCR variability and no evidence of any MOCR activity. The TEOAE-MOCR was normal.

- Participant 128L: there was a systematic increase in MOCR%, most noticeably at 53 dB SPL. The increase was 4.7pp; a rise from 9.3% at 44 dB SPL to 14.0% at 53 dB SPL. The increase at 50 dB SPL was only 1.5pp from 47 dB SPL. The TEOAE-MOCR was high.

- Participant 134R: there was a systematic increase in MOCR%, most noticeably at 53 dB SPL. The increase was 4.8pp; a rise from 12.7% at 44 dB SPL to 17.4% at 53 dB SPL. The increase at 50 dB SPL was only around 1.6pp from the strength at 44 dB SPL. The TEOAE-MOCR was normal.

- Participant 135L: there was a systematic increase in MOCR% but also with a corresponding increase in MOCR variability, so we cannot ascribe the pattern to MOCR activity. The TEOAE-MOCR was normal.

- Participant 140R: the difference at 44 dB SPL is offset by variation elsewhere. There was no systematic increase in MOCR%, so we cannot ascribe the pattern to MOCR activity. The TEOAE-MOCR was normal. This participant provided very inconsistent results and was treated as a special case in subsequent experiments.

It was hypothesized that if the TEOAE elicitor produced a MOCR then the strength of the MOCR would increase with increasing elicitor level. The individual results by the pattern of MOCR strength as a function of stimulus level are as follows:

- Participants 110, 112, 115, 123, 124, 135 (40%) showed no MOCR activity at any level (defined as MOCR ≤10%). Participants 110 and 115 had unknown MOCR strength. Participant 112 also had unknown strength but had very low variability, which may have made any reaction easier to detect. Participants 123, 124, and 135 had normal MOCR strength.

- Participant 140 potentially showed MOCR activity at 44 dB SPL, but this difference is likely to be random as the higher stimulus levels do not show any difference. This participant tended to produce unstable results in other experiments as well.

- Five Participants (117, 134, 108, 119, 128) possibly showed a small difference at 47 dB SPL with MOCR strength greater than variability. Again, two participants (117, 119) did not show MOCR consistently increasing at higher levels, leaving just three ears that did (20%).

- Seven Participants (108, 109, 115, 117, 118, 128, 134) showed a possible difference at 50 dB SPL, but not all were compelling, being very small and not necessarily consistently increasing with level. Only four (27%) of the seven participants (108, 109, 128, and 134) showed further MOCR activity at 53 dB SPL.

- Five Participants (106, 108, 109, 128, and 134) showed a possible difference at 53 dB SPL, with 128 and 134 being the only compelling cases.

- Only three participants (108, 128, and 134) showed a possible systematically increasing difference over all levels in the direction expected (20%).

Could the effects at 53 dB SPL be due to the middle-ear muscle reflex (MEMR) instead? Even a small MEMR response could confound the small MOCR response (Lapsley Miller and Marshall, 2014). Using the MEMR results from the screening day, the MEMR to the 53 dB SPL TEOAE chirp for Participant 128 was below 0 dB SPL, but detectable from variability. For Participant 134, the effect was also at or below 0 dB SPL, but also not particularly distinct from variability. For Participant 109, there was no MEMR at 53 dB SPL. The MEMR could be adding to the observed MOCR at 53 dB SPL, but the evidence was not compelling.

3.2 ANOVA

A repeated-measures ANOVA was carried out on the aggregate MOCR% results with factors Level (44, 47, 50, 53 dB SPL) and Pairing (QN vs. QQ/NN, representing MOCR% and MOCR variability). These eight conditions all met the assumptions for normality as indicated by Shapiro-Wilks tests. It was expected *a priori* that variability would remain constant over the levels and that MOCR% would increase with level or be significantly different from the variability at the higher levels.

The ANOVA showed a significant main effect for Pairings ($F_{1,14}$=15.4, p<0.05) and for Levels ($F_{3,42}$=9.07, p<0.05) and a significant interaction ($F_{3,42}$=2.91, p<0.05, Figure 2). Bonferroni post hoc *t*-tests were used to parse them out (family-wise significance level was p<0.05/16=0.003). The QQ conditions did not differ detectably from one another (six comparisons). Some of the QN conditions differed significantly (six comparisons): MOCR% for the 53 dB SPL elicitor was significantly different from the 44 and 47 dB SPL levels but a significant difference was not detected between 53 dB SPL and 50 dB SPL. Comparing MOCR% and MOCR variability for the same levels (four comparisons), there were no significant differences detected.

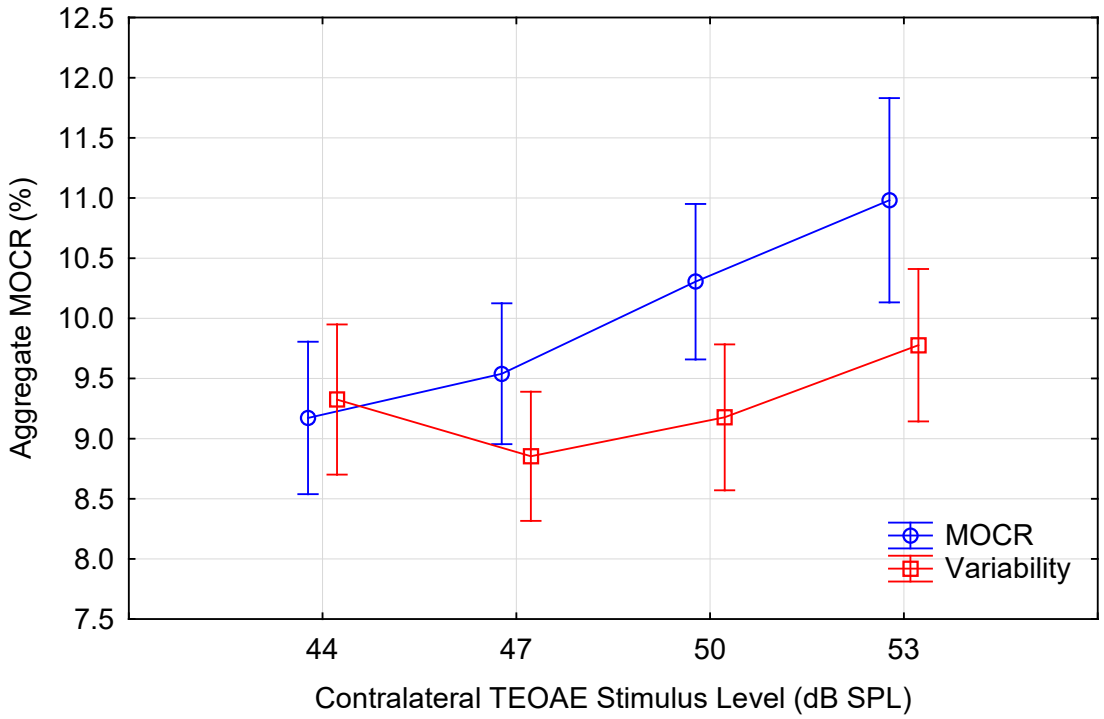

Figure 2. Aggregate MOCR% for the 15 ears compared to the inherent test variability for each contralateral TEOAE stimulus level. Error bars represent the 95% confidence intervals.

3.3 Summary

Results indicate that the TEOAE stimulus at 53 dB SPL might elicit a small MOCR in some ears, but this cannot be wholly disambiguated from a confounding MEMR. Either way, we do not recommend using this TEOAE level for a MOCR assay. Any effect at the lower level of 50 dB SPL is minimal and is possibly not clinically significant. A 50 dB SPL TEOAE stimulus appears unlikely to elicit a confounding MOCR for TEOAE-based MOCR assays, but the slightly lower 47 dB SPL is a more cautious choice. We do not recommend using levels higher than 50 dB SPL.

There are no direct comparisons for this nonlinear TEOAE stimulus that we are aware of in the literature, and as reported by others, the effects of transient elicitors vary widely with their parameters and presentation rates (Guinan *et al.*, 2003). The current results are specific to this assay.

Subsequent to this study, a TEOAE-based MOCR assay was developed using a linear-mode stimulus, where all stimuli are output at the same level, along with different response filtering to remove the artifact (Lapsley Miller *et al.*, in preparation-c). One of the benefits of this new approach is that the higher stimulus in each ensemble that is 9.5 dB higher than the target level is no longer needed. This should reduce the likelihood of the TEOAE stimulus eliciting an ipsilateral MOCR, potentially allowing a higher target stimulus level to be safely used. The current finding is still of interest, however, because a test module is available to researchers on the Mimosa Acoustics HearID system using the published nonlinear mode method (Marshall *et al.*, 2014).

4 References

ANSI/ASA (**2009**). "Methods for Manual Pure-Tone Threshold Audiometry (ANSI S3.21-2004 R2009)," (American National Standards Institute, New York).

Backus, B. C., & Guinan, J. J. (**2007**). "Measurement of the distribution of medial olivocochlear acoustic reflex strengths across normal-hearing individuals via otoacoustic emissions," J. Assoc. Res. Otolaryngol. **8**, 484-496.

Froehlich, P., Collet, L., Valatx, J. L. et al. (**1993**). "Sleep and active cochlear micromechanical properties in human subjects," Hear. Res. **66**, 1-7.

Goodman, S. S., Mertes, I. B., Lewis, J. D. et al. (**2013**). "Medial olivocochlear-induced transient-evoked otoacoustic emission amplitude shifts in individual subjects," J. Assoc. Res. Otolaryngol. **14**, 829-842.

Guinan, J. J., Jr., Backus, B. C., Lilaonitkul, W. et al. (**2003**). "Medial olivocochlear efferent reflex in humans: otoacoustic emission (OAE) measurement issues and the advantages of stimulus frequency OAEs," J. Assoc. Res. Otolaryngol. **4**, 521-540.

Keppler, H., Degeest, S., & Vinck, B. (**2021**). "Short-Term Test-Retest Reliability of Contralateral Suppression of Click-Evoked Otoacoustic Emissions in Normal-Hearing Subjects," J. Speech. Lang. Hear. Res. **64**, 1062-1072.

Lapsley Miller, J. A., Boege, P., Marshall, L. et al. (**2004a**). *Transient-evoked otoacoustic emissions: Preliminary results for validity of TEOAEs implemented on Mimosa Acoustics' T2K measurement system v3.1.3*. NSMRL Technical Report No. 1232, (Naval Submarine Medical Research Laboratory, Groton, CT).

Lapsley Miller, J. A., Boege, P., Marshall, L. et al. (**2004b**). *Stimulus-frequency otoacoustic emissions: Validity and reliability of SFOAEs implemented on Mimosa Acoustics' SFOAE measurement system v2.1.18*. NSMRL Technical Report No. 1231, (Naval Submarine Medical Research Laboratory, Groton, CT).

Lapsley Miller, J. A., & Marshall, L. (**2014**). *Three methods for estimating the middle-ear muscle reflex (MEMR) using otoacoustic emission (OAE) measurement systems*. NSMRL Technical Report No. 1310, (Naval Submarine Medical Research Laboratory, Groton, CT).

Lapsley Miller, J. A., Marshall, L., Reed, C. M. et al. (**in preparation-a**). *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*. NSMRL Technical Report No. xxxx, (Naval Submarine Medical Research Laboratory, Groton, CT).

Lapsley Miller, J. A., Marshall, L., Reed, C. M. et al. (**in preparation-b**). *Effects of stopping rules, stimulus levels, and ear laterality on transient-evoked otoacoustic emission (TEOAE)-based assays of the medial-olivocochlear reflex (MOCR)*. NSMRL Technical Report No. xxxx, (Naval Submarine Medical Research Laboratory, Groton, CT).

Lapsley Miller, J. A., Reed, C. M., Perez, Z. D. et al. (**in preparation-c**). "A clinically-viable medial-olivocochlear reflex (MOCR) assay using linear-mode transient-evoked otoacoustic emissions (TEOAE) on a binaural hardware platform."

Lewis, J. D. (**2018**). "Synchronized Spontaneous Otoacoustic Emissions Provide a Signal-to-Noise Ratio Advantage in Medial-Olivocochlear Reflex Assays," J. Assoc. Res. Otolaryngol. **19**, 53-65.

Lewis, J. D. (**2019**). "The Effect of Otoacoustic Emission Stimulus Level on the Strength and Detectability of the Medial Olivocochlear Reflex," Ear Hear. **40**, 1391-1403.

Liberman, M. C., Liberman, L. D., & Maison, S. F. (**2014**). "Efferent feedback slows cochlear aging," J. Neurosci. **34**, 4599-4607.

Luebke, A. E., & Foster, P. K. (**2002**). "Variation in inter-animal susceptibility to noise damage is associated with alpha 9 acetylcholine receptor subunit expression level," J. Neurosci. **22**, 4241-4247.

Maison, S. F., & Liberman, M. C. (**2000**). "Predicting vulnerability to acoustic injury with a noninvasive assay of olivocochlear reflex strength," J. Neurosci. **20**, 4701-4707.

Maison, S. F., Usubuchi, H., & Liberman, M. C. (**2013**). "Efferent feedback minimizes cochlear neuropathy from moderate noise exposure," J. Neurosci. **33**, 5542-5552.

Marshall, L., & Lapsley Miller, J. A. (**2014**). *How Can The Auditory Efferent System Protect Our Ears From Noise-induced Hearing Loss? Let Us Count The Ways*. (Proceedings of The 12th International Mechanics of Hearing Workshop, Attica, Greece), 23 Jun.

Marshall, L., Lapsley Miller, J. A., Guinan, J. J. et al. (**2014**). "Otoacoustic-emission-based medial-olivocochlear reflex assays for humans," J. Acoust. Soc. Am. **136**, 2697-2713.

Muller, J., Dietrich, S., & Janssen, T. (**2010**). "Impact of three hours of discotheque music on pure-tone thresholds and distortion product otoacoustic emissions," J. Acoust. Soc. Am. **128**, 1853-1869.

Muller, J., & Janssen, T. (**2008**). "Impact of occupational noise on pure-tone threshold and distortion product otoacoustic emissions after one workday," Hear. Res. **246**, 9-22.

Shera, C. A., & Guinan, J. J. (**2003**). "Stimulus-frequency-emission group delay: a test of coherent reflection filtering and a window on cochlear tuning," J. Acoust. Soc. Am. **113**, 2762-2772.

Shupak, A., Tal, D., Sharoni, Z. et al. (**2007**). "Otoacoustic emissions in early noise-induced hearing loss," Otol. Neurotol. **28**, 745-752.

Wolpert, S., Heyd, A., & Wagner, W. (**2014**). "Assessment of the noise-protective action of the olivocochlear efferents in humans," Audiol. Neurootol. **19**, 31-40.

5 APPENDIX A: List of Acronyms and Terms

BBN: broadband noise

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.

Q: a "quiet" trial where no contralateral elicitor was used

QQ/NN: re-paired Q and N trials used to measure the inherent test variability.

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 OAE level between the Q and N trial and is normalized by dividing by the magnitude of the Q trial OAE level.

MOCR variability: like the MOCR derived strength statistic, which is expressed as a percentage, but calculated by taking the vector difference in OAE level between adjacent Q trials and N trials separately (i.e., they are re-paired), and then normalized as above with the first N trial in the pair used as the denominator. This is an estimate of the inherent test variability, which varies considerably from ear to ear.

NSMRL: Naval Submarine Medical Research Laboratory.

N: a "noise" trial where a contralateral elicitor was used

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

pp: percentage points

SFOAE: stimulus-frequency otoacoustic emission – a sound evoked in the inner ear by a tonal stimulus and measured in the ear canal with a sensitive microphone. It is derived by also using a stronger suppressor frequency at a slightly higher or lower frequency than the probe. The suppressor is cycled on and off, and the vector difference at the probe tone is the SFOAE. The SFOAE test can be used as the OAE test in a MOCR assay.

SOAE: synchronized spontaneous otoacoustic emission – a sound generated in the inner ear independent of an evoking stimulus. SOAEs may interfere with SFOAE-based MOCR measurements, so SFOAE test frequencies are selected to avoid them. A TEOAE measurement is used to synchronize the SOAE, allowing its measurement. The measurement is made in a 20ms time window after the TEOAE itself has dissipated.

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 used as the OAE test in a MOCR assay.

Time course: Q1 – (2-second onset) – elicitor – N1 – (10-second offset) – Q2 …

WB: wideband

6 APPENDIX B: Algorithm for selecting SFOAE test frequencies

SFOAE test frequencies for MOCR assays should have sufficient amplitude, not be in a spectral null, and be far enough away from spontaneous otoacoustic emission (SOAE) activity, which can confound SFOAE measurements. These aims can only be achieved by individually selecting test frequencies for individual ears. Here the 59-point SFOAE test, which was run on the screening day, was used to determine the six test frequencies. The 59 points were spread between 1 and 2 kHz, approximately, using the power-law spacing option in the SFOAE-SG software. This spacing ensured that group delay could be calculated without aliasing (Lapsley Miller *et al.*, 2004b). In addition, an SOAE test was run to determine the test frequencies affected by spontaneous activity.

For each potential test frequency, quality control and selection criteria were applied. Many of these criteria came from earlier work, especially Lapsley Miller *et al.* (2004b).

- SFOAE probe and suppressor stimulus levels within 3 dB of target levels of 40/60 dB SPL.
- The difference between (probe-target) and (suppressor-target) had to be less than 3 dB. This accounted for cases where the levels met the 3 dB target above but went in opposite directions (e.g., 42/58 dB SPL was only 2 dB off target but 4 dB from the target difference).
- SFOAE level at least 0 dB SPL.
- SFOAE SNR at least 20 dB SNR.
- Total noise floor less than -5 dB SPL.
- Nearest neighbor SFOAE levels (one frequency bin above and below) within 2.5 dB of SFOAE level, to ensure testing is on a flatter part of the spectrum.
- The correlation coefficient between SFOAE unwrapped phase and frequency had to be less than -0.96 (strong negative slope) for the frequency point to be included (Lapsley Miller *et al.*, 2004b). The unwrapped phase, which is used as a measure of SFOAE validity, was calculated from the cluster of five frequencies centered on the frequency of interest. At least three of the five frequencies needed an SNR of 3 dB, and only those that met this criterion were included in the correlation coefficient calculation.
- Any SFOAE frequencies within one microstructure period from an SOAE frequency were excluded (Shera and Guinan, 2003). Period=$1/((11.1\times F^{0.37})/F)$ in Hz. An SOAE was considered present in a frequency bin if it had SNR > 12.0 dB, Noise < -30.0 dB SPL, and Level > -20.0 dB SPL.

Within any 500 Hz band in the 1-2 kHz frequency range, the aim was to find six frequencies spaced at least 23 Hz apart that passed all quality control and selection criteria. Because the SFOAE spectrum with power-law spacing had only 59 out of the 87 possible frequencies, interpolation was used to estimate the 87-pt spectrum. The quality of the estimated points depended on the status of their nearest neighbors. Every possible 500 Hz band was considered for a solution of six frequencies. If more than six frequencies met selection criteria, those with the highest amplitudes were selected. The final selected sextet used in the experiment was from the band with the highest average SFOAE amplitude.

Not all participants had six test frequencies meeting these criteria and were not therefore included in the experiment.

The following figures (Figure 3, Figure 4, Figure 5, Figure 6) show examples of various solution scenarios.

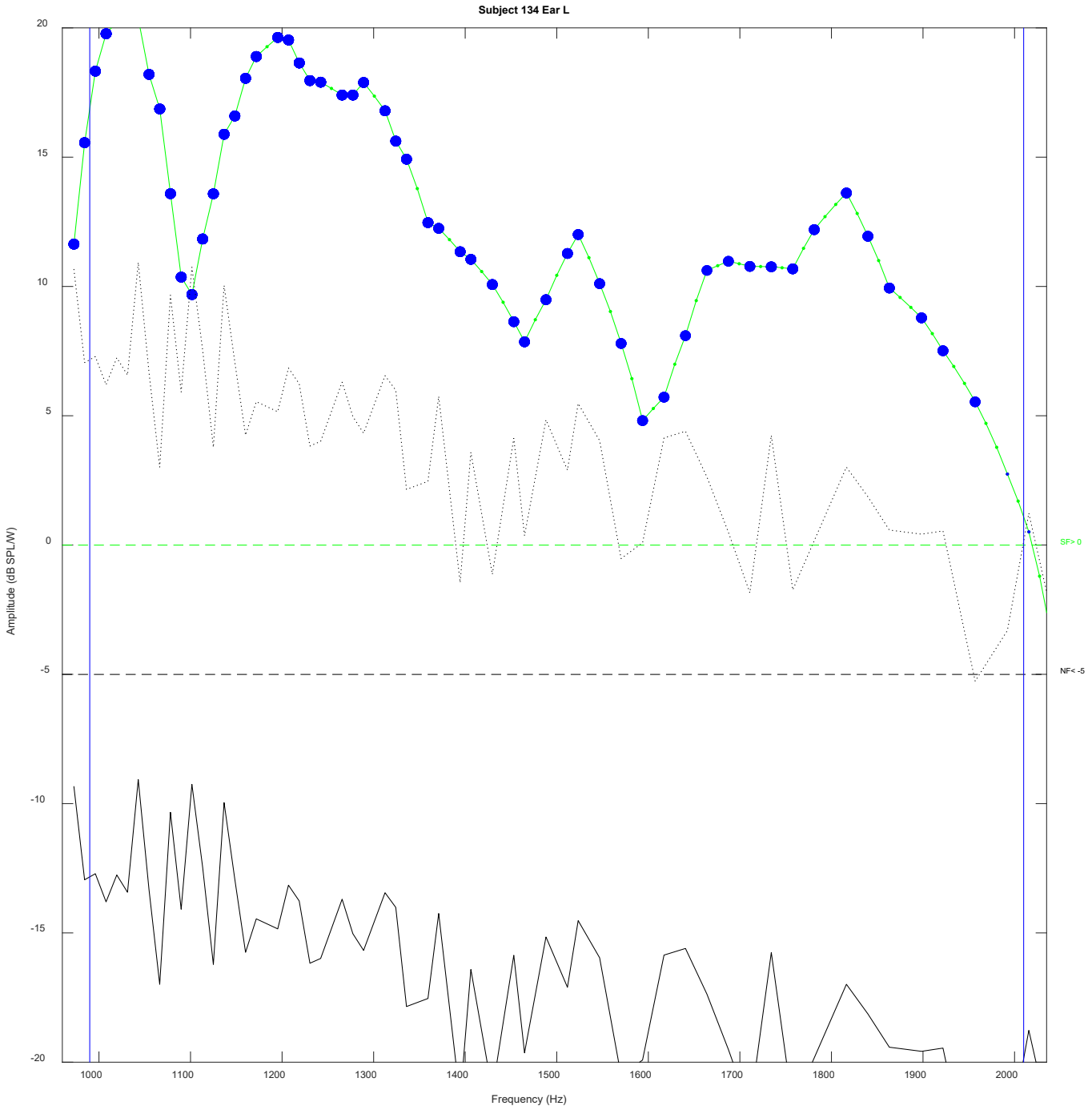

Figure 3. An example where there is no solution due to the presence of SOAEs. The green line is the SFOAE spectrum. The large blue dots are those SFOAEs affected by SOAE activity, defined as within one microstructure period of an SOAE (SOAE themselves not plotted). The black line is the noise floor (it is below the criterion of -5 dB SPL, represented by the horizontal dashed line). The dotted black line indicates the 20 dB SNR criterion (all SFOAE points meet this criterion). The green dashed line represents the minimum SFOAE amplitude (all points pass).

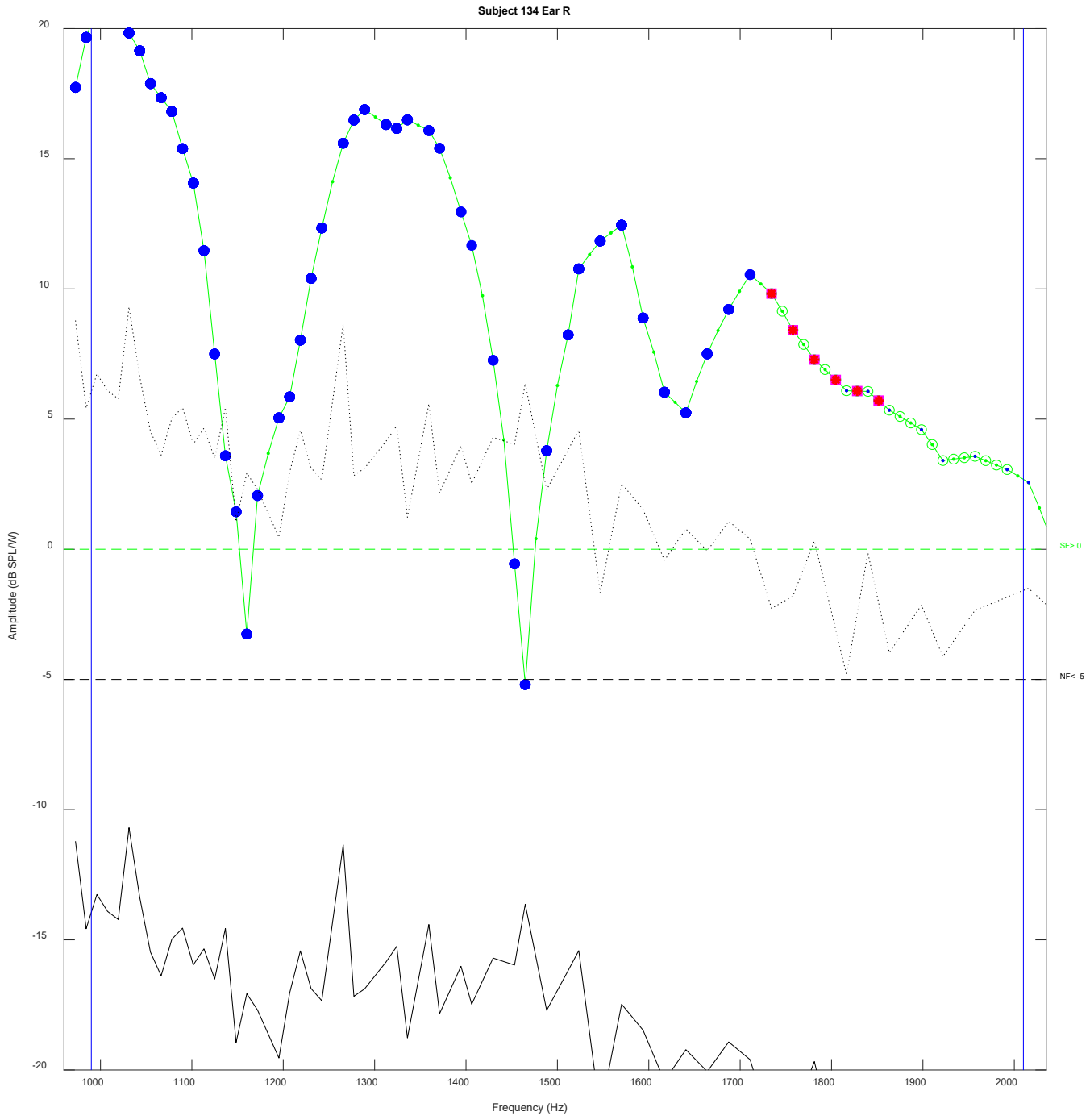

Figure 4. The other ear for participant 134 also had SOAE activity, but above approximately 1.7 kHz, there was a region of frequencies that met all criteria (indicated as red squares). The small blue dots surrounded by green circles indicated other valid frequencies. The green circles without blue dots indicate those frequencies that did not meet at least one criterion (here it would be that they were within 23 Hz of a better frequency). The green line is the SFOAE spectrum. The large blue dots are those SFOAEs affected by SOAE activity, defined as within one microstructure period of an SOAE (SOAE themselves not plotted). The black line is the noise floor (it is below the criterion of -5 dB SPL, represented by the horizontal dashed line). The dotted black line indicates the 20 dB SNR criterion. The green dashed line represents the minimum SFOAE amplitude.

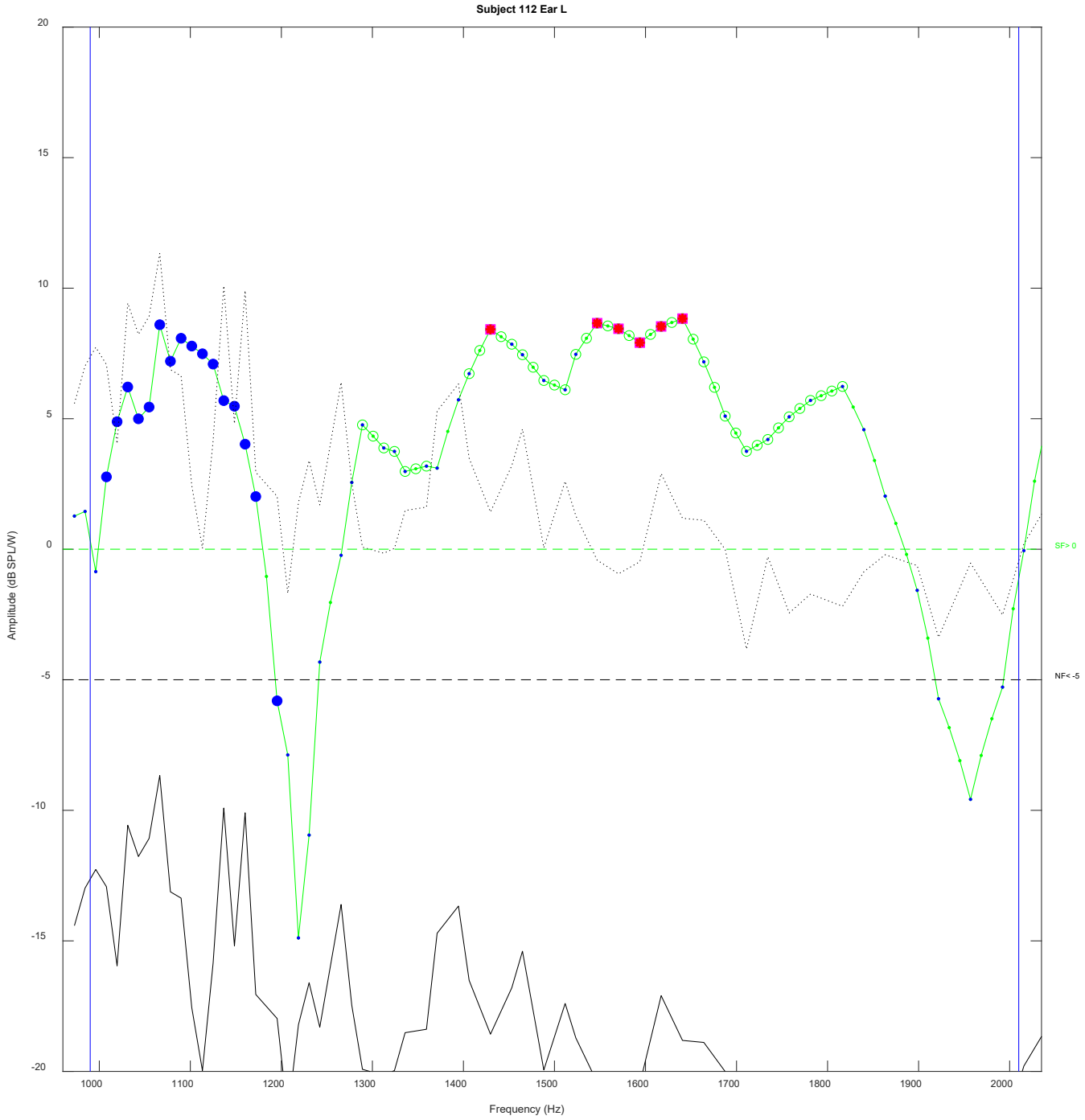

Figure 5. This participant had multiple potential regions with a solution (indicated as red squares). The sextet with the highest average SPL within a 500 Hz band was selected. The small blue dots surrounded by green circles indicated other valid frequencies. The green circles without blue dots indicate those frequencies that did not meet at least one criterion. The green line is the SFOAE spectrum. The large blue dots are those SFOAEs affected by SOAE activity, defined as within one microstructure period of an SOAE (SOAE themselves not plotted). The black line is the noise floor (it is below the criterion of -5 dB SPL, represented by the horizontal dashed line). The dotted black line indicates the 20 dB SNR criterion. The green dashed line represents the minimum SFOAE amplitude.

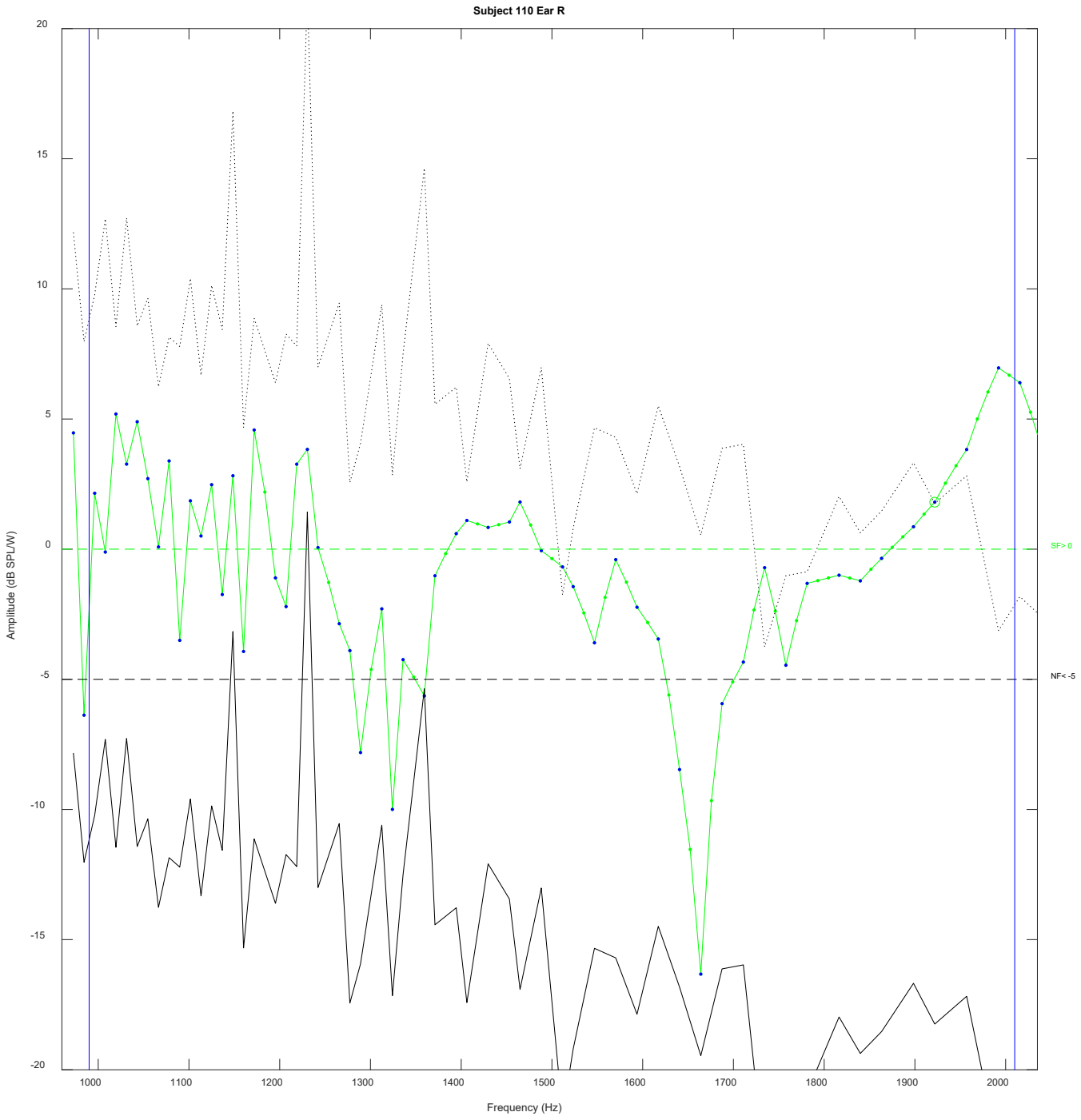

Figure 6. This participant had no solution due to high noise and low SFOAE amplitude. Only one frequency met the screening criteria, indicated by the small blue dot surrounded by a green circle. The green line is the SFOAE spectrum. The black line is the noise floor, which sometimes peaks above the noise floor criterion of -5 dB SPL, represented by the horizontal dashed line. The dotted black line indicates the 20 dB SNR criterion. The green dashed line represents the minimum SFOAE amplitude.