The Effect of Test, Electrode, and Rate on Electrocochleography Measures

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Abstract

Background: Electrocochleography (ECochG) is the measurement of stimulus-related cochlear potentials and the compound action potential (AP). Its primary clinical application is with the assessment of inner ear disorders. There are few studies examining the variability of ECochG measures.

Purpose: The objective of the study was to examine the effect of test (i.e., initial versus retest), electrode (i.e., extratympanic versus tympanic), and stimulus rate (i.e., 7.7 versus 77.7/sec) on ECochG indices (i.e., summating potential [SP] amplitude, AP latency, AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio).

Research Design: Correlational and three-factor repeated measures designs were employed.

Study Sample: Eighteen normal-hearing young adults participated.

Data Collection and Analysis: ECochG responses were obtained with 90 dB nHL click stimuli for an initial test and retest at two stimulus rates with a commercially available extratympanic (TIPtrode™) and tympanic (Lilly TM-Wick) electrode. Separate repeated measures linear mixed-model analysis of variance examined the effect of test, electrode, and rate for all ECochG indices. Test–retest variability was also examined with correlation analyses; an examination of mean test–retest differences and their 95% confidence intervals (CI); and construction of Bland-Altman plots.

Results: The presence of SP and AP responses varied across experimental conditions. Electrode and rate were statistically significant predictors ($p < 0.05$) of SP and AP responses: SP and AP responses were more likely to be present with the tympanic electrode and at the slow rate. Statistically significant correlations ($p < 0.05$) were found between initial tests and retests with all ECochG indices with both electrodes with the exception of SP amplitude with the TIPtrode™ electrode. There were no significant main effects of test (initial versus retest) or interactions of test and electrode or rate for any of the ECochG indices ($p > 0.05$). The 95% CI of the mean test–retest differences contained 0 confirming that the effect of test was not statistically significant. There was a statistically significant main effect of electrode ($p < 0.05$) on three ECochG measures. The Lilly TM-Wick electrode produced larger SP amplitudes, AP amplitudes, and SP/AP area ratios than TIPtrode™ electrodes. A statistically significant main effect of rate ($p < 0.05$) was identified for all ECochG measures. The effect of rate on AP latency and amplitude was expected. Increasing the stimulus rate prolonged the AP latency and decreased AP amplitude. SP amplitude was larger for the faster rate.

Conclusions: There was no difference between electrodes with regard to test–retest measures. However, considering the higher likelihood of ECochG SP and AP responses and larger SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio indices, the tympanic electrode placement is recommended for clinical practice. The addition of a fast stimulus rate may be considered for enhanced SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio albeit with the consideration of the loss of SP and AP responses in some individuals.

Key Words: action potential, electrocochleography, electrode, stimulus rate, summating potential, test–retest variability

Abbreviations: ANOVA = analyses of variance; AP = compound action potential; CI = confidence intervals; ECochG = electrocochleography; SD = standard deviation; SP = summating potential

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INTRODUCTION

Electrocochleography (ECochG) is the measurement of stimulus-related cochlear potentials including the cochlear microphonic, summating potential (SP), and the compound action potential (AP) of the auditory nerve. It is a useful tool in the diagnosis, assessment, and monitoring of inner ear disorders and can be helpful in the diagnosis of retrocochlear disorders. The most common applications for ECochG include diagnosing, assessment, and monitoring of inner ear disease, enhancement of wave I of the auditory brainstem response when hearing loss is present, and measurement and monitoring of auditory nerve function during surgery (Ferraro, 2010).

ECochG has also emerged as one of the more powerful tools in the diagnosis, assessment, and monitoring of Ménière's Disease. A histological marker for Ménière's disease is the presence of hydrops in the endolymphatic space (Gürkov et al, 2016). The classic ECochG presentation of Ménière's disease is an enhanced SP response relative to the AP component (Gibson et al, 1977; Coats, 1981; Ferraro and Durrant, 2006). Unfortunately, the reported incidence of an enlarged SP and SP/AP amplitude ratio in the general Ménière's population is only approximately 60–65%. As a means of making the ECochG more sensitive, Ferraro and Tibbils (1999) combined both amplitude and duration features of the ECochG to measure the "areas" of the SP and AP components.

Even though ECochG is currently a popular clinical method for detecting increases in pressure in the endolymphatic system of the inner ear, there remain many recording challenges including the lack of reliable normative data, electrode selection, and recording standards (Ferraro and Kileny, 2016; Ferraro et al, 2017). One primary technical consideration while recording is the signal-to-noise ratio. ECochG requires a small electrode placed as close to the response generator as possible for the best signal-to-noise ratio. So what electrode should be employed? Most clinics use an extratympanic electrode approach (Ferraro, 2010) for recording the ECochG. One example is a gold foil electrode wrapped around a foam insert (e.g., TIPtrode™) that is placed in the ear canal. This is most comfortable for the patient but results in a significantly smaller magnitude of the response. A compromise in increased magnitude and decreased signal averaging without significant patient discomfort is the use of a "tymptrode" electrode placed on the lateral surface of the tympanic membrane (Ferraro, 2010). Tymptrode electrodes are now commercially available (e.g., Bio-logic TM-ECochGtrode, Natus Medical Incorporated; Lilly TM-Wick Electrode, Intelligent Hearing Systems; and Sanibel™, Sanibel Supply).

Stimulus rate is another parameter of interest in ECochG recording. Ferraro and colleagues (Ferraro and Durrant, 2006; Ferraro, 2010; Ferraro and Kileny, 2016; Ferraro et al, 2017) and others (e.g., Margolis et al, 1992; 1995) have recommended a slow rate of stimulation (i.e., 8.7–11.3/sec). Others (e.g., Gibson et al, 1977; Coats, 1981; Densert et al, 1994; Marangos, 1996; Wilson and Bowker, 2002) have suggested recording with a fast rate of stimulation (i.e., ≥90/sec), in addition to a slow rate. Their rationale has been that the use of a fast stimulus rate fatigues the AP allowing for better visualization of the SP. Ferraro and Durrant (2006) noted, "unfortunately, the use of such fast rates has not proven to be very successful in the clinic, in part because the AP contribution is not completely eliminated and the SP may also be reduced under extreme conditions (e.g., click rates >90/sec)... (and) rapid clicks presented at loud levels tend to be very annoying for patients" (p. 53).

To date, several studies have reported test–retest variability of ECochG. Bergholtz et al (1976), and Densert et al (1994) used transtympanic electrode placement. Mori et al (1981) investigated normal and hearing-impaired ears using an extratympanic silver ball electrode placed on the posterior-superior auditory canal wall within 3 mm of the tympanic membrane. Park and Ferraro (1999) used a tympanic electrode placement with a custom made tymptrode. There has been only one study evaluating the reliability of the TIPtrode™ electrode (Roland et al, 1993). They evaluated 17 normal hearing adults tested repeatedly over 1-week periods averaging 5.3 weeks. Click stimuli were presented at 95 dB nHL at a rate of 9.7/sec. Averages and standard deviation (SD) of SP and AP amplitudes were measured and SP/AP amplitude ratios calculated. Roland et al (1993) reported an average SP/AP amplitude ratio of 0.22 with an SD of 0.06. To the best of our knowledge, there are no studies examining the repeatability of the ECochG measures with any commercially available tympanic electrode. Finally, only one study has examined test–retest variability at a slow and fast rate of stimulation. Densert et al (1994) examined rates of 10 and 90/sec, albeit with a transtympanic electrode.

The purpose of this study was to further examine the test–retest variability of ECochG. It was first of interest to compare a commercially available extratympanic (TIPtrode™) and tympanic (Lilly TM-Wick) electrode. We were also interested in using a slow and fast stimulus rate. Further, all previous studies examining test–retest variability of ECochG did not include a full complement of possible ECochG indices. Specifically, the purpose of this study was to examine the effect of test, electrode, and stimulus rate on ECochG indices (i.e., SP amplitude, AP latency, AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio).

METHODS

Participants

Participants were 18 Caucasian adults with a negative history of loud noise exposure within 48 hr before data collection. They had no significant history of neurological or otological exposure to loud noise in the past 48 hr and/or communication disorders by self-report. Participants ranged in age from 20 to 30 yr (M = 25.2, SD = 2.9; 14 females and four males). All participants had normal bilateral hearing sensitivity defined as pure tone thresholds at octave frequencies from 250 to 8000 Hz ≤ 15 dB HL (American National Standards Institute, 2010). Participants also had normal middle ear function defined as having indices of peak compensated static acoustic admittance, tympanometric width, tympanometric peak pressure, and equivalent ear canal volume within the 90% range of gender-specific normative data (Roup et al, 1998).

Apparatus

ECochG data acquisition was performed using the Intelligent Hearing Systems SmartEP (Version 3.98) evoked potential system. ECochG responses were evoked with 90 dB nHL 100 μsec click stimuli of alternating polarity. Stimulus intensities were calibrated relative to the behavioral thresholds of ten normal-hearing young adults (Stuart et al, 1990). The reference level (0 dB nHL) for the click was 32 dB peak-to-peak peSPL as assessed in a HA2 2-cm^3 coupler (Brüel & Kjær DB-1038), sound level meter (Brüel & Kjær 2231), and pressure condenser microphone (Brüel & Kjær 4144).

Procedure

The East Carolina University and Medical Center Institutional Review Board approved this research study before data collection or participant recruitment. All participants signed informed consent before data collection. ECochGs were obtained for the initial test and repeat test for both Lilly TM-Wick and TIPtrode™ electrodes. Participants were comfortably seated in a recliner in a quiet examination room during testing. Before testing, otoscopy was performed to ensure that there was not excessive cerumen in the ear canal that would prevent proper electrode placement. Before data collection, Signa-Gel® Electrode Gel was applied to the Lilly TM-Wick electrodes. These were then soaked in a saline solution for 10 minutes. Participants were instructed to sit quietly with little movement throughout each test. A horizontal recording montage was used with the noninverting electrode on the lateral surface of the tympanic membrane for recording with Lilly TM-Wick electrodes or the lateral external auditory canal for TIPtrode™ recordings, the inverting electrode on the contralateral mastoid, and the ground electrode on the high forehead (F_{pz}). The TIPtrode™ electrode was inserted so that the distal edge of the electrode was flush with the entrance to the external auditory meatus. Lilly TM-Wick placement was verified by having the participant report when they heard the electrode bump against the tympanic membrane at which time the electrode lead was carefully taped anteroinferior to the intertragal notch and held while an insert earphone was inserted in the same manner as the TIPtrode™. Interelectrode impedances were kept at or below 7,000 Ω when testing with Lilly TM-Wick electrodes and at or below 1,000 Ω for TIPtrode™ electrodes. Electrode impedances were examined after electrode placement and between recordings. The recorded electroencephalogram was amplified 100,000 times and bandpass-filtered (10 to 1500 Hz). The analysis time was 5.0 msec poststimulus onset. Each recording contained 1,024 samples that were averaged and replicated twice for each rate of 7.7 and 77.7/sec. Test conditions (i.e., two electrodes and two rates) were counterbalanced across participants according to a digram-balanced Latin squares design (Wagenaar, 1969). One ear of each participant was tested. The test ear was counterbalanced across participants. All participants received all ECochG tests in a single session. Each participant received a minimum of eight recordings (i.e., two electrodes × two rates × two replications). In some recording conditions, additional one or two recordings were collected where waveform component identification was difficult. All electrodes were discarded after each test and replaced with a new one for retest.

Electrophysiological Waveform Analysis

The second author, who was blinded to test conditions, analyzed the waveforms. Analyses of wave components were undertaken from the summed replicated waveforms in each condition. The SP waveform component was analyzed in terms of amplitude and the AP waveform component was analyzed in terms of amplitude and latency. The SP/AP amplitude ratio and SP/AP area ratio were also calculated and analyzed. The baseline of the response was identified at the onset of the initial negative deflection of the SP, and the AP was the first negative going peak after 1 msec (Ferraro and Tibbils, 1999; Ferraro, 2010). The SP amplitude was determined from the baseline to the leading edge of the AP. AP amplitude was measured from the baseline to the component trough. The SP/AP area ratio was calculated in the Intelligent Hearing Systems SmartEP system in accordance with recommendation from the manufacturer by marking the amplitude of the base at the point in time following the AP trough where

the response passed through the initial baseline amplitude (Intelligent Hearing Systems, n.d.). Figure 1 illustrates an example analysis of an ECochG recording.

RESULTS

Each participant received four ECochG tests; however, not all generated all identifiable wave SP and AP components. Examples of one participant's recordings are illustrated in Figure 2. Table 1 shows the percentage of ECochG responses as a function of test, electrode, and rate. We undertook logistic regression analyses to examine predictor values of test, electrode, and rate for SP and AP response presence or absence. The analyses revealed that electrode, *Wald statistic* (1) = 10.85, $p = 0.001$, and rate, *Wald statistic* (1) = 14.18, $p < 0.001$, were statistically significant predictors of an SP response. It was also found that electrode, *Wald statistic* (1) = 11.35, $p = 0.001$, and rate, *Wald statistic* (1) = 13.61, $p < 0.001$, were statistically significant predictors of an AP response. That is, SP and AP responses were more likely to be present when recorded with a Lilly TM-Wick electrode and at a slow rate of 7.7/sec.

The test–retest reliability of ECochG with two separate electrode types was examined in four ways. First, Pearson's product-moment correlation coefficients (r) were determined to examine the association between initial test and retest of the five ECochG indices for both electrode types. Second, five separate three-factor linear mixed-model analyses of variance (ANOVA) with repeated measures were performed to determine the effect of test, electrode, and rate on SP amplitude, AP latency, AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio. This ANOVA model can accommodate missing data in a repeated-measures design. The repeated measures were modeled with an autoregressive (order 1) covariance metric. The choice of the covariance structure was based on goodness of fit statistics (i.e., −2 Res Log Likelihood, Akaike's information criterion, Hurvich and Tsai's Criterion, Bozdogan's Criterion, and Schwarz's Bayesian Criterion). Third, because of limitations of the correlation and statistical significance to assess reliability (Bland and Altman, 1986), mean test–retest differences and their 95% confidence intervals (CI) were also examined for each electrode with all five ECochG indices. Finally, Bland-Altman plots (Bland and Altman, 1986; 1999) were constructed to examine reliability between the initial and subsequent tests for all five ECochG indices for both electrode types and two rates. Each Bland–Altman plot is a bivariate scatterplot of the difference of two test measurements (i.e., initial test and retest) on the Y-axis and the average of the two test measurements (i.e., initial test and retest) on the X-axis. Three horizontal reference lines are superimposed on each plot. They include the average difference between the two test measurements (i.e., the bias) and the mean difference ±1.96 SD (i.e., the 95% limits of agreement). Systematic variation with the mean differences of the two measurements should not be evident in the plots. Proportional/systematic bias was explored by examining the differences of two test measurements in each plot with separate t-tests for paired samples. Next, linear trends between the differences of two test measurements were examined with linear regression analysis.

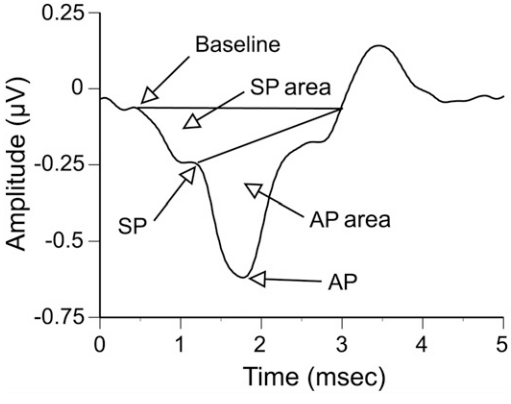

Figure 1. A representative ECochG recording from one participant with identified components of interest (i.e., baseline, SP, AP, SP area, and AP area).

SP Amplitude

Mean and SD for SP amplitudes as a function of test, rate, and electrode are shown in Table 2. There was a statistically significant correlation between the initial test and retest SP amplitudes for the Lilly TM-Wick ($r = 0.53, p = 0.001$) but not for the TIPtrode™ electrode ($r = 0.41, p = 0.06$). A three-factor linear mixed-model repeated-measures ANOVA was conducted to examine SP amplitude differences as a function of test, rate, and electrode. (With this and all subsequent three-factor linear mixed-model repeated-measures ANOVA, a fixed factorial model was first used. When all interactions were not statistically significant [$p > 0.05$], the analysis was then repeated with a fixed main effects model. In all analyses, the findings were the same and therefore, the values from the fixed main effects model were reported.) Statistically significant main effects of electrode, $F_{(1,107.20)} = 17.82$, $p < 0.0001$, and rate $F_{(1,55.22)} = 55.22$, $p = 0.01$, were found. SP amplitudes were significantly larger for the Lilly TM-Wick electrode and for the faster rate of 77.7/sec. The main effect of test and all other interactions were not statistically significant ($p > 0.05$). Means and SD for SP amplitude differences (i.e., initial test–retest) as a function of rate and electrode are shown in Table 3. Also contained in the table are the 95% CI of the mean differences. As evident in

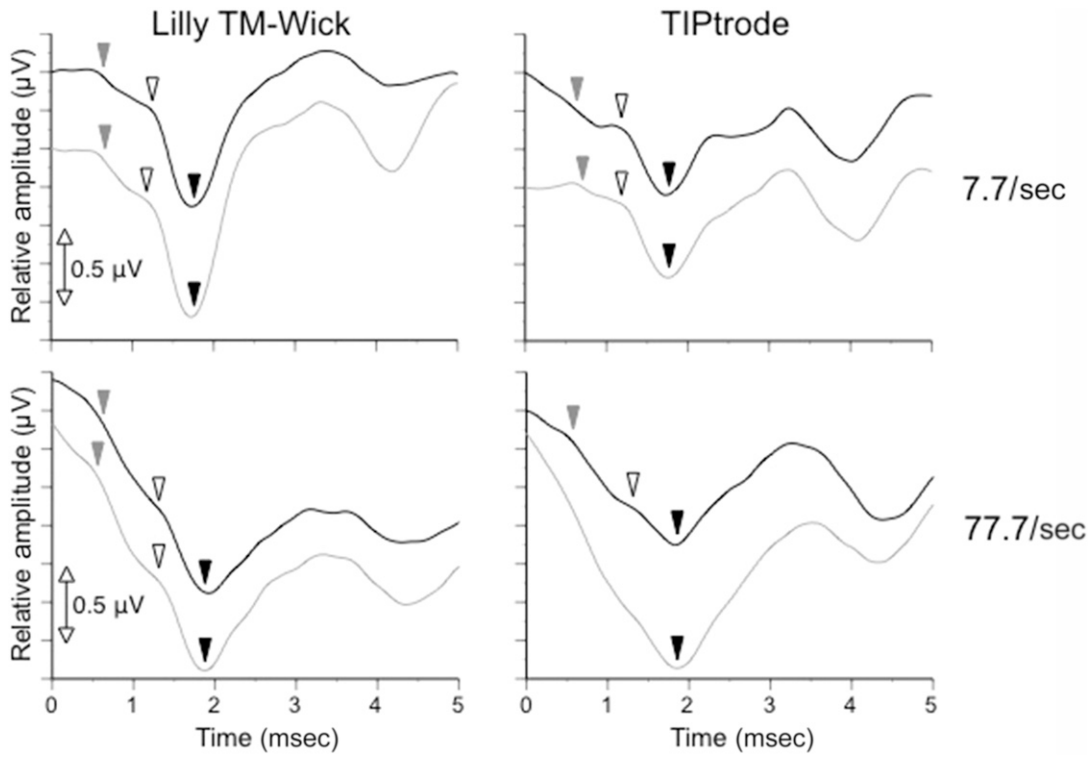

Figure 2. Representative ECochG recordings from one participant as a function of the electrode (i.e., Lilly TM-Wick and TIPtrode™) and rate (i.e., 7.7 vs. 77.7/sec). Gray, white, and black inverted triangles identify baseline, SP, and AP components, respectively.

Table 3, all CI contain 0. This is additional evidence that the effect of test is not statistically significant and SP amplitude measures across tests are reliable. The Bland–Altman plots for SP amplitude as a function of electrode and rate are shown in Figure 3. Two observations are evident in these plots: 95% limits of agreement are larger for the Lilly TM-Wick electrode and the fast stimulus rate. There was no systematic variation with the mean differences of the two measurements evidenced by a nonsignificant difference between test means and no linear predictive relationships between averaged and difference scores in any plot ($p > 0.05$).

Table 1. Percentage of ECochG Responses (%) as a Function of Test, Electrode, and Rate

		Initial Test		Retest	
		ECochG Component		ECochG Component	
Electrode	Rate	SP	AP	SP	AP
Lilly TM-Wick	7.7/sec	100%	100%	94%	100%
	N	18	18	17	18
	77.7/sec	89%	89%	89%	89%
	N	16	16	16	16
TIPtrode™	7.7/sec	94%	94%	94%	94%
	N	17	17	17	17
	77.7/sec	44%	44%	56%	61%
	N	8	8	10	11

AP Latency

Mean and SD for AP latencies as a function of test, rate, and electrode are shown in Table 2. There were statistically significant correlations between initial test and retest AP latencies for both the Lilly TM-Wick ($r = 0.84, p < 0.001$) and TIPtrode™ electrodes ($r = 0.53$, $p = 0.01$). A three-factor linear mixed-model repeated-measures ANOVA was conducted to examine AP latency differences as a function of test, rate, and electrode. A statistically significant main effect of rate, $F_{(1,74.21)} = 147.81, p < 0.0001$, was found. AP latencies were significantly longer for the faster rate of 77.7/sec. The main effects of test and electrode and all interactions were not statistically significant ($p > 0.05$). Means, SD, and 95% CI of the mean AP latency differences (i.e., initial test–retest) as a function of rate are shown in Table 3. All CI contain 0, confirming that the effect of test was not statistically significant, and AP latency measures across tests are reliable. The Bland–Altman plots for AP latency as a function of electrode and rate are shown in Figure 4. The 95% limits of agreement are very similar between the two electrodes and across the two stimulus rates. There was no systematic variation with the mean differences of the two measurements supported by a nonsignificant difference between test means and no linear predictive relationships between averaged and difference scores ($p > 0.05$) in any plot except for the TIPtrode™ Test 1 and 2 with the 7.7/sec rate. An outlier is evident in the plot.

Table 2. Mean ECochG Indices and SD as a Function of Test, Rate, and Electrode

		SP Amplitude (μV)		AP Latency (msec)		AP Amplitude (μV)		SP/AP Amplitude Ratio		SP/AP Area Ratio	
		Test		Test		Test		Test		Test	
Electrode	Rate	Initial	Retest	Initial	Retest	Initial	Retest	Initial	Retest	Initial	Retest
Lilly TM-Wick	7.7/sec	0.28	0.29	1.71	1.70	0.87	1.02	0.32	0.32	0.54	0.52
		(0.17)	(0.21)	(0.13)	(0.11)	(0.37)	(0.59)	(0.14)	(0.15)	(0.17)	(0.19)
		[18]	[17]	[18]	[18]	[18]	[18]	[18]	[17]	[18]	[17]
	77.7/sec	0.37	0.38	1.89	1.86	0.76	0.75	0.51	0.47	0.72	0.71
		(0.25)	(0.37)	(0.14)	(0.12)	(0.45)	(0.53)	(0.17)	(0.18)	(0.16)	(0.19)
		[16]	[16]	[16]	[16]	[16]	[16]	[16]	[16]	[16]	[16]
TIPtrode™	7.7/sec	0.13	0.12	1.70	1.68	0.50	0.51	0.26	0.25	0.40	0.42
		(0.07)	(0.05)	(0.11)	(0.07)	(0.18)	(0.17)	(0.12)	(0.11)	(0.20)	(0.14)
		[17]	[17]	[17]	[17]	[17]	[17]	[17]	[17]	[17]	[17]
	77.7/sec	0.23	0.25	1.86	1.80	0.52	0.43	0.49	0.48	0.64	0.70
		(0.28)	(0.16)	(0.07)	(0.11)	(0.41)	(0.26)	(0.28)	(0.20)	(0.26)	(0.15)
		[8]	[10]	[8]	[11]	[8]	[11]	[8]	[10]	[8]	[10]

Note: Values enclosed in parentheses represent 1 SD of the mean. Values enclosed in brackets represent the sample number.

When this outlier was removed from the analyses, the linear regression was not statistically significant.

AP Amplitude

Mean and SD for AP amplitude as a function of test, rate, and electrode are shown in Table 2. There were statistically significant correlations between initial test and retest AP amplitudes for both the Lilly TM-Wick ($r = 0.39$, $p = 0.02$) and TIPtrode™ electrodes ($r = 0.61$, $p = 0.002$). A three-factor linear mixed-model repeated-measures ANOVA was conducted to examine AP amplitude differences as a function of test, rate, and electrode. Statistically significant main effects of electrode, $F_{(1,112.72)} = 35.74$, $p < 0.001$, and rate $F_{(1,81.18)} = 6.52$, $p = 0.01$, were found. AP amplitudes were significantly larger for the Lilly TM-Wick electrode and for the slower rate of 7.7/sec. The main effect of test and all interactions were not statistically significant ($p > 0.05$). Means, SD, and 95% CI of the mean differences for AP amplitude differences (i.e., initial test–retest) as a function of rate and electrode are shown in Table 3. As evident in Table 3, all CI contain 0, giving additional evidence that the effect of the test is not statistically significant and AP amplitude measures across tests are reliable. The Bland–Altman plots for AP amplitude as a function of electrode and rate are shown in Figure 5. As with SP amplitude, AP amplitude 95% limits of agreement are much larger with the Lilly TM-Wick electrode. There was also no systematic variation with the mean differences of the two measurements demonstrated by a nonsignificant difference between test means and no linear predictive relationships between averaged and difference scores in any plot ($p > 0.05$).

SP/AP Amplitude Ratio

Mean and SD for the SP/AP amplitude ratio as a function of test, rate, and electrode are shown in Table 2. There were statistically significant correlations between the initial test and retest SP/AP amplitude ratios for both the Lilly TM-Wick ($r = 0.49$, $p = 0.003$) and TIPtrode™

Table 3. Mean ECochG Indices Differences, SD, and 95% CI as a Function of Rate and Electrode

Electrode	Rate	SP Amplitude (μV)	AP Latency (msec)	AP Amplitude (μV)	SP/AP Amplitude Ratio	SP/AP Area Ratio
Lilly TM-Wick	7.7/sec	0.00	0.01	−0.15	0.01	0.02
		(0.21)	(0.05)	(0.67)	(0.15)	(0.19)
		[−0.11, 0.11]	[−0.02, 0.04]	[−0.48, 0.18]	[−0.07, 0.08]	[−0.08, 0.12]
	77.7/sec	−0.01	0.02	0.02	0.02	0.01
		(0.36)	(0.11)	(0.11)	(0.17)	(0.17)
		[−0.20, 0.18]	[−0.04, 0.08]	[−0.04, 0.08]	[−0.06, 0.11]	[−0.08, 0.10]
TIPtrode™	7.7/sec	0.00	0.02	0.00	0.01	−0.02
		(0.08)	(0.07)	(0.16)	(0.13)	(0.19)
		[−0.03, 0.04]	[−0.02, 0.06]	[−0.09, 0.08]	[−0.05, 0.08]	[−0.11, 0.08]
	77.7/sec	0.02	0.04	0.08	−0.033	−0.12
		(0.26)	(0.12)	(0.29)	(.23)	(0.16)
		[−0.25, 0.29]	[−0.07, 0.15]	[−0.19, 0.36]	[−0.03, 0.20]	[−0.29, 0.04]

Note: Values enclosed in parentheses represent 1 SD of the mean and values in brackets are lower and upper bounds of the 95% CI.

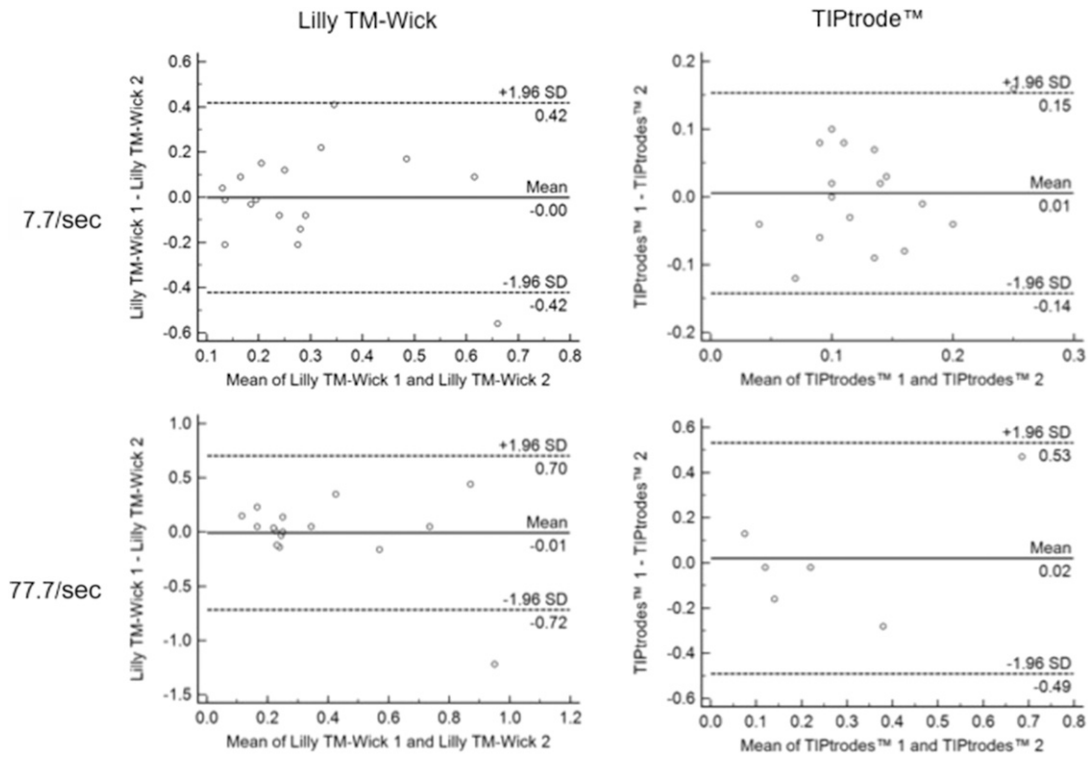

Figure 3. Bland–Altman plots for SP amplitude as a function of electrode and rate. The X- and Y-axis represent the average of the initial and retest SP amplitude measurements and difference of the two, respectively. Solid lines represent average mean test difference. Broken lines represent ±2 SD (i.e., limits of agreement).

electrodes ($r = 0.52$, $p = 0.01$). A three-factor linear mixed-model repeated-measures ANOVA was conducted to examine the SP/AP amplitude ratio differences as a function of test, rate, and electrode. A statistically significant main effect of rate, $F_{(1,68.28)} = 73.95$, $p < 0.001$, was found. SP/AP amplitude ratios were significantly larger for the faster rate of 77.7/sec. The main effect of test and electrode and all interactions were not statistically significant ($p > 0.05$). Mean, SD, and 95% CI of the mean differences for SP/AP amplitude ratio differences (i.e., initial test–retest) as a function of rate and electrode are shown in Table 3. All CI contain 0, giving additional evidence that the effect of test is not statistically significant and SP/AP amplitude ratio measures across tests are reliable. The Bland–Altman plots for the SP/AP amplitude ratio as a function of electrode and rate are shown in Figure 6. The SP/AP amplitude ratio 95% limits of agreement are similar between the two electrodes and stimulus rates. A nonsignificant difference between test means and no linear predictive relationships between averaged and difference scores in any plot ($p > 0.05$) confirmed that there was no systematic variation with the mean differences of the two measurements.

SP/AP Area Ratio

Mean and SD for SP/AP area ratio as a function of test, rate, and electrode are shown in Table 2. There were statistically significant correlations between the initial test and retest SP/AP amplitude area ratios for both the Lilly TM-Wick ($r = 0.49$, $p = 0.003$) and TIPtrode™ electrodes ($r = 0.50$, $p = 0.02$). A three-factor linear mixed-model repeated-measures ANOVA was conducted to examine SP/AP area ratio differences as a function of test, rate, and electrode. Statistically significant main effects of electrode, $F_{(1,108.20)} = 10.80$, $p = 0.001$, and rate $F_{(1,68.28)} = 78.44$, $p < 0.001$, were found. SP/AP area ratios were significantly larger for the Lilly TM-Wick electrode and for the faster rate of 77.7/sec. The main effect of test and electrode and all interactions were not statistically significant ($p > 0.05$). Means, SD, and 95% CI of the mean differences for SP/AP area ratio differences (i.e., initial test–retest) as a function of rate and electrode are shown in Table 3. As with all other ECochG indices, all CI contain 0, attesting to the fact that the effect of test is not statistically significant and SP/AP amplitude area ratio measures across tests are reliable. The Bland-Altman plots for the SP/AP area ratio as a function of electrode and rate are shown in Figure 7. The SP/AP area ratio 95% limits of agreement were similar between the two electrodes at the slow stimulus rate. At the fast rate, however, the Lilly TM-Wick had a much larger 95% limits of agreement versus the TIPtrode™ electrode. There was no systematic variation with the mean differences of the two measurements evidenced by a nonsignificant difference

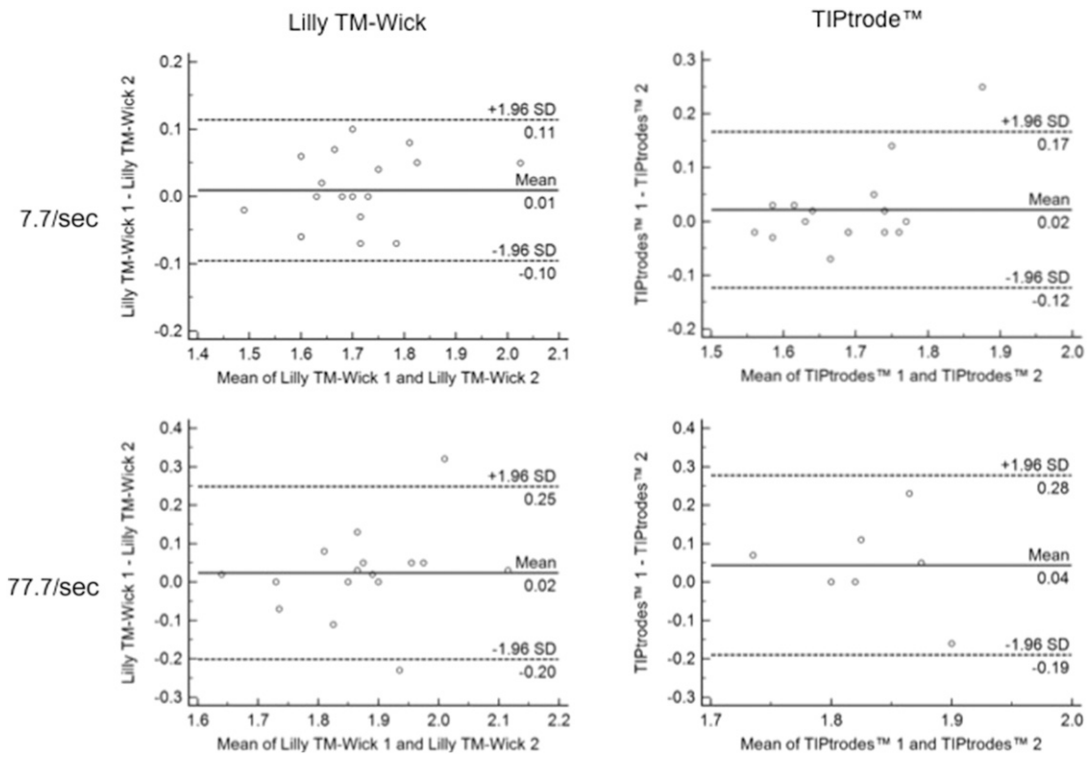

Figure 4. Bland–Altman plots for AP latency as a function of electrode and rate. The X- and Y-axis represent the average of the initial and retest AP latency measurements and difference of two, respectively. Solid lines represent average mean test difference. Broken lines represent ±2 SD (i.e., limits of agreement).

between test means and no linear predictive relationships between averaged and difference scores in any plots ($p > 0.05$).

DISCUSSION

The main purpose of the study was to examine the effect of test–that is, test–retest variability on ECochG indices (i.e., SP amplitude, AP latency, AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio) using commercially available extratympanic and tympanic electrodes and two stimulus rates. This is the first report of reliability of ECochG indices including the SP/AP area ratio recorded with extratympanic (TIPtrode™) and tympanic (Lilly TM-Wick) electrodes at slow (7.7/sec) and fast (77.7/sec) stimulus rates. We employed a number of statistical means to accomplish this. First, we found statistically significant correlations between initial tests and retests with all ECochG indices with both electrodes with the exception of SP amplitude with the TIPtrode™ electrode. Second, we conducted separate three-factor linear mixed-model repeated-measures ANOVAs to examine the effect of test on all ECochG indices. There were no significant main effects of test (initial versus retest) or interactions of test and electrode or rate for any of the ECochG indices. Further, with each ECochG index, the 95% CI of the mean test–retest contained 0, confirming that the effect of test was not statistically significant. Finally, Bland–Altman plots (Bland and Altman, 1986; 1999) were constructed to examine absolute reliability between the initial test and retest. There was no systematic variation with the mean differences of the two measurements and no linear predictive relationships between averaged and difference scores across all ECochG indices. Also, if test measures were repeatable, one would expect the mean test differences to be zero and 95% of the differences to be less than ±1.96 SD. This was apparent on all plots. Considering all analyses, one can conclude that ECochG measures are reliable with both the Lilly TM-Wick and TIPtrode™ electrodes, across the initial test and retest performed over a short period of time, in young adult participants. We agree with Eason (1991) in that, "reliability is a characteristic of data, albeit data generated on a given measure administered with a given protocol to given subjects on given occasions" (p. 84).

There was a statistically significant main effect of electrode on three ECochG measures. The Lilly TM-Wick electrode produced larger SP amplitudes, AP amplitudes, and SP/AP area ratios than TIPtrode™ electrodes. These findings are similar to others with regard to SP and AP amplitudes (Ruth and Lambert, 1989; Ferraro et al, 1994; Ferraro and Krishnan,

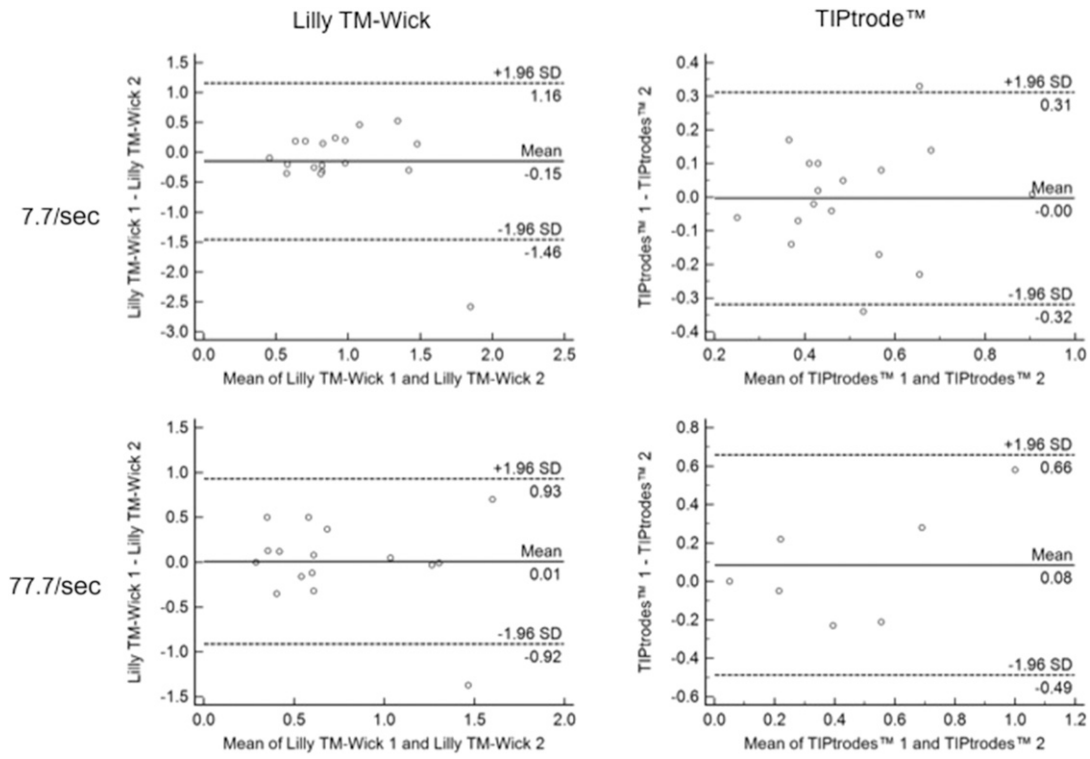

Figure 5. Bland–Altman plots for AP amplitude as a function of electrode and rate. The X- and Y-axis represent the average of the initial and retest AP amplitude measurements and difference of the two, respectively. Solid lines represent average mean test difference. Broken lines represent ±2 SD (i.e., limits of agreement).

1997; Ferraro, 2010). This electrode effect on these amplitude measures can be attributed to the electrode's closer location to the ECochG generators. The nonsignificant difference in AP latencies between electrodes was expected.

A statistically significant main effect of the rate was identified for all ECochG measures. The effect of the rate on AP latency and amplitude was expected. Increasing the stimulus rate prolonged the AP latency and decreased AP amplitude. SP amplitude was larger for the faster rate. This is consistent with the findings of Wilson and Bowker (2002). They reported that SP amplitude increased with click stimulus rate increasing from 7.1 to 51.1/sec and then plateauing at 101.1 and 151.1/sec. The findings are also similar to increased SP amplitude with increased stimulus rate observed with ECochGs to tonal stimuli (Wuyts et al, 2001). The concomitant increase in SP amplitude and decrease in AP amplitude can be attributed for the statistically significant increase in SP/AP amplitude ratios and SP/AP area ratios with an increase in stimulus rate from 7.7 to 77.7/sec.

SP amplitude, AP latency, AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio are generally similar to those reported in the literature for extratympanic (Coats, 1981; Oh et al, 2014) and tympanic (Margolis et al, 1992; Ferraro and Tibbils, 1999; Grasel et al, 2017) electrode placements. Variability across measures was also similar for extratympanic (Roland et al, 1993) and tympanic (Park and Ferraro, 1999) electrode placements. Our findings with regard to rate are similar to Wilson and Bowker (2002) in that a slow rate allowed for easily identified ECochG components. They reported that "at high stimulus rates, the ECochG morphology degraded, and significant ($p < 0.05$) changes occurred to all ECochG components, but primarily to the AP, SP/AP ratio and waveform width" (p. 515). They also observed the absence of a response in some participants. Initially, the logistic regression analyses were used to examine the predictor values of test, electrode, and rate for the presence or absence of a response. The findings were consistent with the notion that ECochG SP and AP responses are more likely to be present when recorded at a slower stimulus rate of 7.7/sec than a faster rate of 77.7/sec.

Several researchers have reported the presence/absence of ECochG components in large-scale normative studies. Oh et al (2014) used an extratympanic electrode while recording ECochGs in 30 normal adults ranging in age from 21 to 63 yr ($M = 43.5$ yr). Details of the extratympanic electrode were not provided. Responses were evoked with 95 dB nHL click stimuli presented at a rate of 9.1/sec. SP and AP components were identified in 100% of ears tested. Wilson and Bowker

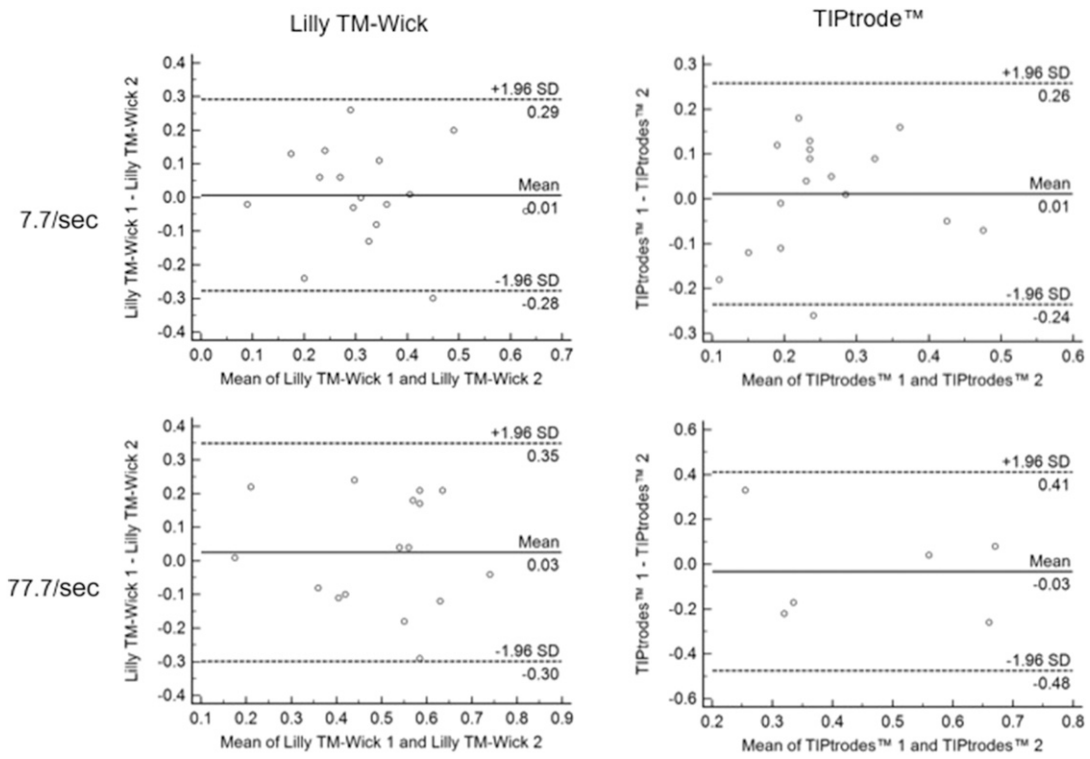

Figure 6. Bland–Altman plots for SP/AP amplitude ratio as a function of electrode and rate. The X- and Y-axis represent the average of the initial and retest SP/AP amplitude ratio measurements and difference of two, respectively. Solid lines represent average mean test difference. Broken lines represent ±2 SD (i.e., limits of agreement).

(2002) reported ECochG findings in 102 ears from 51 normal-hearing adults aging from 19 to 71 yr. They recorded ECochGs with a tympanic electrode. Responses were evoked with 90 dB nHL click stimuli presented at rates of 7.1, 51.1, 101.1, and 151.1/sec. At the slowest rate, SP and AP components were identified in 100% of ears. With increasing rates, the numbers of participants exhibiting ECochG components decreased slightly: At a rate of 51.1/sec, 99% of ears displayed SPs and APs. SPs were present in 94% and 96% of ears at rates of 101.1 and 151.1/sec, respectively. APs were present in 91% and 93% of ears at rates of 101.1 and 151.1/sec, respectively. Grasel et al (2017) examined 200 ears from 100 normal-hearing adults aged from 19 to 71 yr (M = 43.6 yr). ECochGs were recorded with a tympanic electrode evoked by 90 dB nHL click stimuli presented at a rate of 11.3/sec. They evidenced APs in 100% of ears and SPs in 64% of ears. The variability of the ECochG component expression between the present studies and previous ones can be attributed to differences in electrodes, stimulus rates, participants' ages, and sampling distributions.

More variability was evidenced with ECochG amplitude measures as opposed to the latency measure. That is, greater variability was seen in SP and AP amplitudes versus AP latency as evidenced in greater variance across measures (see Table 2) and between initial and retest measures illustrated by mean differences (see Table 3), and the Bland–Altman plots (Figures 3 and 5). This is not surprising considering the latency of the AP response is time-locked to stimulus onset. By contrast, SP and AP amplitudes are contingent on the averaged true evoked response and the residual background noise. Because residual noise varies from sweep to sweep, greater variability in SP and AP amplitudes is expected across repeated measures. More variability in SP and AP amplitudes was noted with the Lilly TM-Wick electrode compared to the TIPtrode™ electrode. It may have been the case that greater residual noise was evidenced during the Lilly TM-Wick electrode recording. One limitation of the study design was not simultaneously recording two channel recordings from the extratympanic (TIPtrode™) and tympanic (Lilly TM-Wick) locations. If that were the case, differences between variability in SP and AP amplitudes could exclusively be attributed to the electrode location. One could also argue that higher and less stable electrode impedances, which are typical for tympanic electrodes (Durrant, 1986; Ferraro, 2010), make them more susceptible to noise during recording. Durrant (1986), however, found no statistically significant correlation between AP amplitude and tympanic electrode impedance. The greater variability in SP and AP amplitudes with the tympanic Lilly TM-Wick electrode can likely be attributed to variability in placement relative to closer

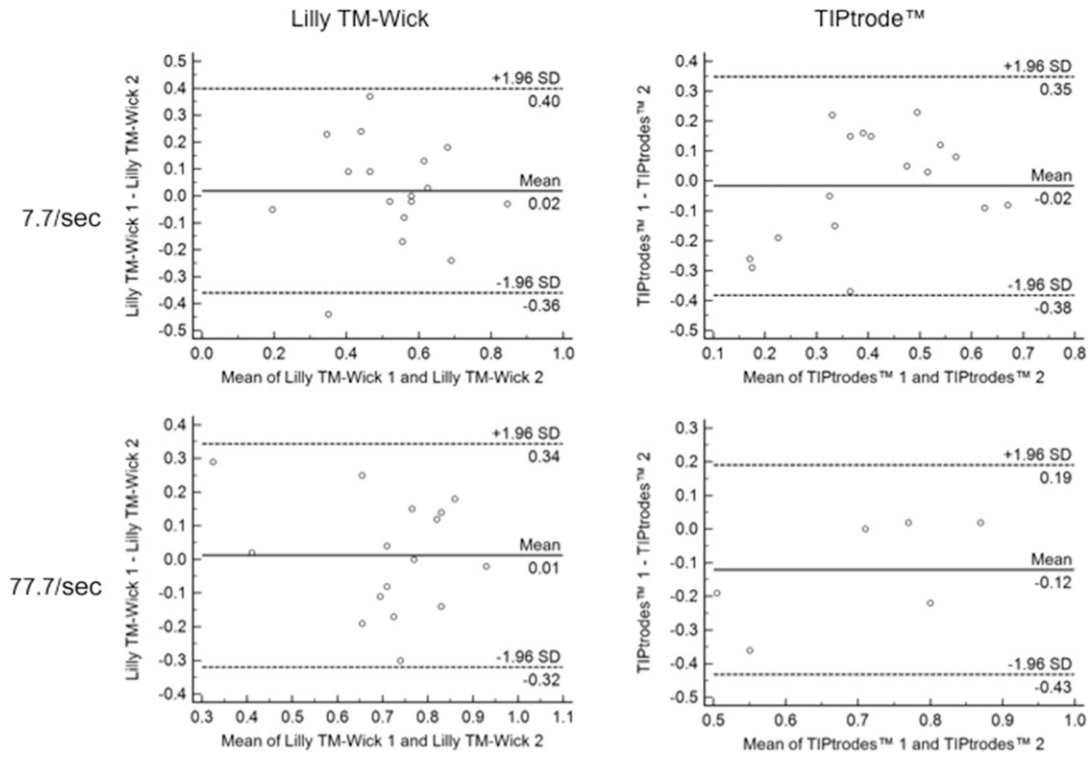

Figure 7. Bland–Altman plots for the SP/AP area ratio as a function of electrode and rate. The X- and Y-axis represent the average of the initial and retest SP/AP area ratio measurements and difference of two, respectively. Solid lines represent average mean test difference. Broken lines represent ±2 SD (i.e., limits of agreement).

proximity to the generator sources. With the extratympanic electrode being afforded a more distant placement from the generator sources and a larger area of interface with the canal, there should be less variability across individuals and between repeated measures in the same ear during far-field recording. With the tympanic Lilly TM-Wick electrode being located closer to the generator sources, it is more susceptible to amplitude measure fluctuations with movements across individuals and between repeated measures in the same ear in the near-field versus far-field recording. This is also exacerbated with a smaller contact area for the Lilly TM-Wick electrode. An additional issue with our Lilly TM-Wick electrode was that the placement was not done under direct otoscopic/oto-microscopic visualization to confirm that the electrode tip was against the tympanic membrane. Although the Lilly TM-Wick placement was verified by having participants report that they heard the electrode bump against the tympanic membrane, the electrode might have bumped against the ear canal and/or moved before being taped to the intertragal notch, during placement of the insert earphone, and/or during recording. The noise floor can increase when the electrode tip is not in contact with the tympanic membrane (Ferraro, 2010).

These findings are of interest with the clinical application of ECochG measures and the choice of the electrode and the stimulus rate. First, these findings are consistent with the notion that ECochG recordings with both the extratympanic (TIPtrode™) and tympanic (Lilly TM-Wick) electrode placements are reliable tests to be used in assessment and reassessment of ECochG indices. The tympanic Lilly TM-Wick electrode, however, has an advantage in the significant higher likelihood of observing ECochG SP and AP responses. The logistic regression analyses identified electrode as a significant predictor of both SP and AP responses. Further, SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio were significantly larger with the Lilly TM-Wick electrode. Considering the higher likelihood of ECochG SP and AP responses and larger SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio indices, we recommend the tympanic electrode placement. The disadvantage of the tympanic electrode placement is greater variability (as noted above) in SP and AP amplitude measures. Clinicians may consider using a fast rate in addition to recording ECochGs with a slow rate. The fast rate produced significantly higher SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio. This suggestion has been voiced previously as a means to better visualize the SP response (Gibson et al, 1977; Coats, 1981; Densert et al, 1994; Marangos, 1996; Wilson and Bowker, 2002). Finally, clinicians may use the Bland–Altman plots to determine if the test–retest variation is acceptable for their clinical practice. Ninety-five percentage of test–retest differences were less than ±1.96 SD. The magnitude of these bounds varied, however, across electrode

type, stimulus rate, and ECochG indices (see Figures 1–5). The magnitudes of these bounds were small enough that the measures could be considered clinically reliable.

In conclusion, we examined the effect of the repeated test, electrode (i.e., extratympanic [TIPtrode™] and tympanic [Lilly TM-Wick]), and stimulus rate (i.e., slow and fast) on ECochG indices including SP amplitude, AP latency, AP amplitude, SP/AP amplitude ratio, and SP/AP area ratio. We also explored whether test, electrode, and rate were predictor values for SP and AP response presence or absence. It was found that SP and AP responses were more probable to be present when recorded with a Lilly TM-Wick electrode and at a slow rate of 7.7/sec. The test–retest reliability of ECochG with two separate electrode types was tested with four measures (i.e., correlation coefficients, linear mixed-model ANOVA, mean test–retest differences, and Bland–Altman plots). Statistically significant correlations were found between initial tests and retests with all ECochG indices with both electrodes with the exception of SP amplitude with the TIPtrode™ electrode. There was no difference between electrodes with regard to test–retest measures. However, considering the higher likelihood of ECochG SP and AP responses and larger SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio indices, our clinical recommendation is to use the tympanic electrode placement. The addition of a fast stimulus rate, during clinical practice, may be considered for enhanced SP amplitude, SP/AP amplitude ratio, and SP/AP area ratio, albeit with the consideration of the loss of SP and AP responses in some individuals. Finally, we concur with Ferraro and colleagues' (Ferraro and Kileny, 2016; Ferraro et al, 2017) call for the standardization of ECochG recording and measurement protocols. This includes some consensus across manufactures of ECochG systems in the measurement of SP and AP areas (cf. Figure 1 above with the Intelligent Hearing Systems SmartEP [Version 3.98] and Figure 1 from Grasel et al (2017) with the Interacoustics Eclipse Otoaccess [Version 1.2.1]). It would behoove audiology-training programs to adopt such standards and train their students accordingly. This would include instruction and clinical practice with the use of otoscopic/oto-microscopes for visualization of the external auditory canal, tympanic membrane, and confirmation of tympanic electrode placement against the tympanic membrane. Proper instruction and clinical training of students will in turn assure patient comfort and consistent and reliable clinical ECochG recordings.

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