Research Article

Stability of the Cortical Sensory Waveforms, the P1-N1-P2 Complex and T-Complex, of Auditory Evoked Potentials

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Purpose: Atypical cortical sensory waveforms reflecting impaired encoding of auditory stimuli may result from inconsistency in cortical response to the acoustic feature changes within spoken words. Thus, the present study assessed intrasubject stability of the P1-N1-P2 complex and T-complex to multiple productions of spoken nonwords in 48 adults to provide benchmarks for future studies probing auditory processing deficits.

Method: Response trials were split (split epoch averages) for each of 4 word types for each subject and compared for similarity in waveform morphology. Waveform morphology association was assessed between 50 and 600 ms, the time frame reflecting

spectro-temporal feature processing for the stimuli used in the study.

Results: Using approximately 70 trials in each split epoch, the P1-N1-P2 complex was found to be highly stable, with high positive associations found for all subjects for at least 3 word types. The T-complex was more variable, with high positive associations found for all subjects to at least 1 word type.

Conclusions: The P1-N1-P2 split epochs at group and individual levels and the T-complex at group level can be used to assess consistency of neural response in individuals with auditory processing deficits. The T-complex relative to the P1-N1-P2 complex in individuals can provide information pertaining to phonological processing.

The phoneme sequence within a spoken word is composed of a series of spectro-temporal features. The cortical sensory waveform responses of the auditory evoked potentials (AEPs), the P1-N1-P2 complex and T-complex, reflect processing of the spectro-temporal feature changes within spoken words (Martin & Boothroyd, 2000; Ostroff, Martin, & Boothroyd, 1998; Wagner et al., 2016) and, therefore, have potential for probing deficits in auditory processing. In this study, we investigated intra-subject stability of these cortical sensory AEP responses.

The cortical sensory waveforms of the AEPs and their neuromagnetic counterparts within the auditory evoked fields (AEFs) have been identified as markers of auditory processing deficits associated with language impairment, dyslexia, and autism (Bishop, Hardiman, Uwer, & Suchodoletz,

2007; Giraud et al., 2005; Guttorm, Leppänen, Hämäläinen, Eklund, & Lyytinen, 2010; Hämäläinen, Fosker, Szucs, & Gowami, 2011; Roberts et al., 2010; Seery, Vogel-Farley, Tager-Flusberg, & Nelson, 2013; Shafer, Schwartz, & Martin, 2011; Wible, Nicol, & Kraus, 2002). For example, Roberts et al. (2010) identified delayed mN100 responses of the AEF to tones ranging in frequency from 200 to 1000 Hz in children with autism relative to a control group, and only the children with typical development showed the expected reduction in mN100 latency associated with increasing age (Ponton, Eggermont, Khosla, Kwong, & Don, 2002). The authors argued that the delayed mN100 response in children with autism reflects deficits in encoding auditory information.

The P1-N1-P2 and T-complex waveforms of the AEP are electrical signals obtained from the scalp surface at fronto-central (FC) and posterior temporal (PT) electrode sites, respectively (for review, see Steinschneider, Liegeois-Chauvel, & Brugge, 2011). These AEP responses reflect synchronous activity within neural ensembles in response to sound, and the morphology of the sensory waveform (i.e., the timing and amplitude of the series of peaks and valleys) is

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modulated by the characteristics of the sound (e.g., frequency, intensity, and duration; Martin, Tremblay, & Stapells, 2007). P1-N1-P2 and T-complex responses are elicited by each acoustic feature change within a spoken word. Hence, the sensory responses to a series of acoustic feature changes within the phoneme sequence overlap (Martin & Boothroyd, 2000; Ostroff et al., 1998) to form a waveform pattern that is unique for each spoken word (Wagner, Shafer, Martin, & Steinschneider, 2013; Wagner et al., 2016). These waveform patterns might be used to probe deficits in auditory processing by determining whether spectral and temporal features within spoken words are recognized within auditory cortex. The P1-N1-P2 complex has been shown to reflect primarily acoustic-level processing of the speech signal (Elangovan & Stuart, 2011; Sharma & Dorman, 2000; Wagner et al., 2013, 2016), and the superior temporal plane is its primary source (Steinschneider et al., 2011). The T-complex has been traced to activity within the posterior lateral superior temporal gyrus (Howard et al., 2000; Steinschneider et al., 2011) and has been shown to represent acoustic processing, as well as feature integration for phonological processing (Chang et al., 2010; Friedrich, Schild, & Roder, 2009; Mesgarani, Cheung, Johnson, & Chang, 2014; Wagner et al., 2013). Evaluation of the P1-N1-P2 and T-complex waveforms in individuals with auditory processing deficits can also provide information pertaining to the origin of the deficit, specifically, whether the deficit originates within the superior temporal plane or the posterior lateral superior temporal gyrus.

In individual subjects, the P1-N1-P2 and T-complex responses are obtained by averaging multiple single-response trial epochs that are time-locked and phase-locked to the stimulus. Variations within these single trial responses that differ will be eliminated with averaging. Atypical cortical sensory responses (i.e., averaged responses) obtained from individuals with auditory processing deficits may be caused by a consistent absence of a synchronous neural response to an acoustic feature change within the speech. It is possible, however, that a synchronous neural response occurs to an acoustic feature, but without sufficient frequency in the single-response trials to be evident after averaging. Hence, it is possible that atypical sensory waveform patterns that have been found in individuals with impaired auditory processing resulted from an increased level of variation in neural response. Analysis of single trials is necessary to determine consistency of neural responses to speech stimuli (Gilley & Sharma, 2010). We are unaware of studies that have examined variability of single-response trials to speech at cortical levels in individuals with auditory processing deficits, although studies examining auditory processing at the level of the brain stem have found increased variability of neural responses in individuals with poor reading skills relative to individuals with average and good reading skills (Hornickel & Kraus, 2013).

Giraud et al. (2005) examined the cortical sensory AEP responses from scalp electrodes at the FC and temporal brain regions and demonstrated that four individuals with dyslexia failed to respond to the burst for /b/ within the syllable /ba/ following French prevoicing relative to

individuals without dyslexia. The individuals without dyslexia showed the initial sensory response to the prevoicing and a second sensory response to the burst for the /b/ within the syllable /ba/. The cortical sensory responses from the subjects with dyslexia suggest an absence of a synchronous neural response to the acoustic feature change (i.e., the burst for /b/) within the syllable /ba/ that followed the prevoicing. While not discussed in the study, it is also possible that the subjects with dyslexia responded to the acoustic feature change (i.e., the burst for /b/) within the syllable /ba/, but without sufficient consistency across single-response trials to show the typical waveform pattern after averaging.

Trial-to-trial variability of cortical-level responses to an auditory stimulus, even an identical stimulus, occurs in subjects without auditory processing deficits (Bernasconi et al., 2011). However, the amount of variability within single-trial cortical sensory responses to natural speech for language learners with typical development has not yet been established. Waveforms created by averaging multiple single-trial responses reflect phase-locked activity. Single-trial variability may be caused by induced non-phase-locked neural activity, which may include disturbances in the ongoing electroencephalogram (EEG) rhythms (see Gilley & Sharma, 2010) or external noise in the environment. Single-trial variability may also be a result of cellular noise (random fluctuations) at all levels of the nervous system (for review, Faisal, Selen, & Wolpert, 2008). For example, as described by Faisal et al., spontaneously released neurotransmitters from presynaptic neurons can cause postsynaptic changes in current. These postsynaptic current changes might then affect the response to a subsequent neural signal (stimulus). Bernasconi et al. (2011) questioned whether cellular-level fluctuations could affect perception of a stimulus presented at threshold levels. They instructed subjects to identify tone stimuli as having either high or low pitch (or short or long duration), even though the presented stimuli were identical in pitch (or duration). As expected, behavioral perceptions of pitch (or duration) were at chance levels. Single-trial AEP sensory responses, however, predicted erroneous perceptions for a majority of subjects. The authors argued that random fluctuations within neurons resulted in the erroneous perceptions of the stimuli at the single-trial level.

Considering the interest in cortical sensory responses of the AEPs and AEFs for probing deficits in auditory processing (Guttorm et al., 2010; Hämäläinen et al., 2011; Roberts et al., 2010) and for investigating neural plasticity following exposure to speech and speech training (Sheehan, McArthur, & Bishop, 2005; Tremblay, Inoue, McClannahan, & Ross, 2010; Tremblay & Kraus, 2002), determining the stability of the P1-N1-P2 complex and T-complex in response to speech that varies in a naturalistic manner is necessary for future comparisons in research. Tremblay, Friesen, Martin, and Wright (2003) examined intrasubject reliability of P1-N1-P2 responses to single productions of four naturally produced consonant-vowel syllables (i.e., /bi/, /pi/, /si/, and /shi/). Stability of response

was examined in seven adult subjects between two testing sessions that occurred within 8 days of each other. The subjects heard 300 presentations of each syllable during each testing session. The P1-N1-P2 complex was found to be highly stable between the two testing sessions, with inter-class correlations ranging from .60 to .94 in individual subjects. We are unaware of studies that have examined intrasubject stability for the P1-N1-P2 complex in response to multiple exemplars of natural speech and are not aware of studies that have examined intrasubject stability for the T-complex in response to natural speech stimuli. Hence, this investigation of consistency of single-trial cortical sensory responses to multiple productions of spoken nonwords will provide necessary benchmarks for future studies examining stability of cortical sensory responses in individuals with auditory processing deficits.

The findings of Tremblay et al. (2003) led us to hypothesize that P1-N1-P2 split epoch waveform responses to multiple productions of spoken words would be highly similar at the single-subject level. Greater intersubject variability for the T-complex relative to the P1-N1-P2 complex waveforms has been previously reported (Bishop, Hardiman, & Barry, 2012); thus, we predicted that split average epochs for the T-complex would be only moderately associated at the single-subject level. Furthermore, using naturally varying speech acoustics, we explored whether approximately 70 single trials would be sufficient to obtain signature P1-N1-P2 and T-complex waveform patterns that reflect grand mean patterns, which are necessary for probing spectro-temporal feature processing in individuals with auditory processing deficits.

Method

Subjects

Forty-eight adults between the ages of 21 and 39 years (mean age = 29 years; 12 men, 36 women) were tested. Participants were native English or native Polish speakers without a history of speech and language, reading, or academic difficulty as indicated by personal report. Hearing acuity was screened at 25-dB hearing level (HL) at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz and found to be within normal limits for all subjects. Four of the subjects were left-handed. Two groups of 24 subjects (12 Polish and 12 English speakers in each group) were tested as part of other studies using experimental methods differing slightly for each group (group 1 and group 2), as detailed below. These same subjects were examined in an earlier study (for details, see Wagner et al., 2016).

Stimuli

In total, 560 nonsense word pairs were presented. For the current study, the AEP response to the first word in the word pairs was analyzed. Spoken nonsense words were produced by a male, English–Polish bilingual speaker, with English being his dominant language. The nonsense words contained the phoneme sequence onsets /st/, /sæt/, /pt/,

and /pæt/. Each phoneme sequence onset (e.g., /st/) occurred in 35 nonwords (e.g., “steema,” “stila”) that were produced twice for a total of 70 natural productions. Phoneme sequences that followed the onsets /st/, /sæt/, /pt/, and /pæt/ (e.g., “eema,” “ila”) varied, and word types were matched for rhyme (e.g., /stima/, /ptima/, /sætima/, /pætima/). All nonwords were potential real words in the Polish and English languages with the exception of /pt/, which does not occur in word onset in English.

These 70 natural productions for each word type occurred within the first word of a same pair and within the first word of a different pair for a total of 140 stimuli per word type. Thus, each of the four word types was presented 140 times for a total of 560 presentations. Two subjects in group 1 and four subjects in group 2 were presented with additional productions of each word type (maximum 20 productions), presented randomly from the 140 stimuli. An interstimulus interval of 250 ms separated words in the word pairs, and the intertrial interval was 2 s.

Our previous work showed that the P1-N1-P2 and T-complex waveforms between 50 and 600 ms corresponded to processing the phoneme sequence onsets (i.e., /st/, /sæt/, /pt/, and /pæt/), and AEP responses to the subsequent phoneme sequences (the rhyme) that varied were cancelled with averaging (see Wagner et al., 2016). Thus, the mean durations (and ranges) for the phoneme sequence onsets measured from word onset to the burst for /t/ were as follows: /pæt/ 149 ms (123 to 174 ms), /pt/ 114 ms (84 to 140 ms), /sæt/ 258 ms (210 to 325 ms), and /st/ 208 ms (159 to 259 ms).

Procedure

The EEG was recorded while subjects listened to the nonsense word pairs and performed a syllable identification task for the second word in the word pairs. Participants were required to count the syllables within the second word in the pair to determine whether they could distinguish (behaviorally) the native and nonnative phoneme sequence contrasts (see Wagner, Shafer, Martin, & Steinschneider, 2012).

To assess intrasubject stability of P1-N1-P2 and T-complex responses to the first word within the pairs, we created waveforms from each half of the single-trial responses (split epoch average, approximately 70 trials) from the total of approximately 140 single-trial responses to each of the four word types (i.e., /st/, /sæt/, /pt/, and /pæt/) for each individual. We subsequently determined the association between these split epoch average waveforms and each word type for each individual. AEP epochs for each word type (e.g., /st/) included in the split epoch average were time-locked to either the first word of the same pairs or the first word of the different pairs. All same and different word pairs within the total stimulus set of 560 word pairs were randomized and separated into seven blocks, and these seven blocks were presented to subjects in random order. Thus, same and different word pairs for all word types were presented randomly, and subjects were unaware of whether the word was within a same or different pair

upon presentation of the first word in the pair. The mean number of accepted single trials (and range) in each split epoch average for each word type for the 48 subjects is listed in Table 1.

In our previous studies, the P1-N1-P2 and T-complex morphology to both native and nonnative phoneme sequence onsets was found to be highly similar in native English and native Polish listeners (Wagner et al., 2013, 2016); therefore, subjects within Polish and English language groups were combined to obtain grand mean split epoch waveforms in the current study.

EEG Data Acquisition

Testing procedures differed slightly for the two groups of 24 subjects. Testing occurred in a sound-treated electrically shielded room, and stimuli were presented using E-prime software (version 1.1; Psychology Software Tools, 2002). Words were presented in the free field through either overhead speakers at altitudes of 30° and 80° relative to the subject's head (Realistic Minimus-7; group 1) or through left and right speakers at an azimuth of 45° from the subject's head (Realistic Minimus-7; group 2). Stimuli were equalized for root mean square amplitude and played at 55.5-dB sound pressure level (group 1) or 62.5-dB sound pressure level (group 2).

EEG Data Processing

EEG data were acquired using a 65-channel net (Electrical Geodesics Inc., Eugene, OR). Sampling rate was 250 Hz for group 1 or 500 Hz for group 2, and data were bandpass filtered between 0.1 and 30 Hz (group 1) or between 0.1 and 100 Hz and low-pass filtered at 30 Hz using Net station (version 4.5.1; group 2). The EEG was referenced to the vertex electrode (Cz). Data were processed using BESA Research (version 5.3.7, Brain Electrical Source Analysis [BESA], Germany). Eye-blink and ocular-movement artifacts were detected and removed using an adaptive correction function (Ille, Berg, & Scherg, 2002). Data were then scanned for remaining artifacts using amplitude and signal gradient criteria. If an excessive number of trials from a channel contained artifacts, data from that channel were interpolated using spherical spline interpolation (Perrin, Pernier, Bertrand, & Echallier, 1989). Epochs were segmented 100 ms pre-word onset and 800 ms post-word onset, and data were re-referenced for average referencing. Single trials were exported without averaging.

Statistical Analysis of EEG

The P1-N1-P2 complex was defined as amplitude values recorded from FC electrode sites 4 (near Fz in the

10–20 system), 5 (near FC1), 55 (near FC2), and vertex electrode Cz between 50 and 600 ms. The T-complex was defined as amplitude values recorded from left and right PT sites 24 (near T7 in the 10–20 system) and 52 (near T8) between 50 and 600 ms. The time interval 50 to 600 ms was found to correspond to sensory processing for the stimuli used in the study (Wagner et al., 2013). P1-N1-P2 split epoch stability was assessed at FC site 4 (near Fz) and averaged FC sites, and T-complex split epoch stability was assessed at left PT site 24 (near T7) and right and left averaged PT sites.

Method of Statistical Analysis

The Pearson product-moment correlation coefficient was calculated (Microsoft Excel software; Microsoft, Redmond, WA) to assess the association between split epoch averages for the P1-N1-P2 complex and T-complex in response to each word type for each subject and to assess the association between the grand average P1-N1-P2 complex and T-complex split epochs to each word type for groups 1 and 2. Pearson r was used, rather than interclass correlation, because we were interested in stability of waveform morphology, irrespective of amplitude, which represents spectro-temporal feature processing for the spoken word (Wagner et al., 2016). The number of data points between 50 and 600 ms for group 1 was 139 ($df = 137$), critical value $r = .278$ ($p < .001$), and that for group 2 was 278 ($df = 276$), critical value $r = .197$ ($p < .001$).

Results

P1-N1-P2 split epoch averages, including approximately 70 single-trial responses to multiple productions of natural speech (e.g., /st/ word types) within each average, were highly similar for individual subjects and reflected the grand mean patterns. T-complex split epoch averages were moderately similar for individual subjects; however, all subjects showed T-complex split epoch waveforms that were highly similar for at least one word type.

Stability of the P1-N1-P2 Complex to Multiple Productions of Natural Speech

P1-N1-P2 grand mean split epoch waveform responses to each word type were almost perfect replicas ($r = .99$), demonstrating that approximately 70 trials provided excellent representation of the sensory processing in individual subjects. This finding is further supported because highly similar patterns of deflection to each word type were found

Table 1. The mean number and range of accepted single-response trials in each split epoch average for each word type for the 48 subjects.

Parameter	Word type							
	/pet/		/pt/		/set/		/st/	
Mean	68	68	68	68	68	68	69	68
Range	57–79	59–78	51–84	58–80	54–81	59–79	56–82	60–82

from two independent groups of subjects (see center graphs within Figure 1).

Table 2 provides the mean (and range) of Pearson r values for each word type from 48 subjects, demonstrating highly stable P1-N1-P2 waveforms from individual subjects using approximately 70 single-trial responses to multiple productions of spoken words. Table 2 also shows the percentages of subjects found to have a significant positive association between split epoch averages ($p < .001$) to each word type. All subjects' P1-N1-P2 split epoch waveforms at averaged FC sites were found to have a significant positive association for the /sət/, /pət/, and /st/ word onsets (100%), and all except two out of 48 subjects (96%) showed a significant positive association for split epoch averages to the /pt/ onsets ($p < .001$). More importantly, Table 3 illustrates that all subjects' r values reflect high positive associations (i.e., $r > .50$) between split epochs at FC sites for at

least three word types (Pearson $r < .50$, highlighted in bold). Results shown in Tables 2 and 3, and values at FC site 4 (near Fz) shown in the Supplemental Material, provide baseline measures for stability of P1-N1-P2 waveform responses to natural speech in adult subjects.

Figure 1 illustrates sample split epoch P1-N1-P2 waveform responses to the /st/ onsets in individual subjects at averaged FC sites that surround the grand mean P1-N1-P2 (also to the /st/ onset). The waveforms created from one half of the single trials to the word type (split epochs) are displayed in red color, and the waveforms created from the remaining split epochs are displayed in blue color. The Pearson r values revealing the association between the red and blue waveforms appear above each graph. Waveforms between 50 and 600 ms are shown and correspond to the time interval used for obtaining the correlation coefficients. Sample individual subject waveform patterns from

Figure 1. The grand mean P1-N1-P2 split epoch waveform responses to the /st/ onset at fronto-central (FC) sites (center). Sample split epoch P1-N1-P2 waveform responses to the /st/ onset at FC sites from individual subjects surround the center grand mean P1-N1-P2 graphs. Group 1 sample subject waveforms are shown on the left, and group 2 sample subject waveforms are shown on the right. Waveforms between 50 and 600 ms are shown, which correspond to the time interval used to obtain the Pearson r values. Amplitude scales for individual subject waveforms vary to best illustrate waveform morphology. Note that group 2 data include additional data points, therefore, colors appear darker. Red = split epoch, half of trial responses to /st/ onset; blue = split epoch, remaining half of single trial responses to /st/ onset.

Table 2. The mean and range of Pearson *r* values for individual subjects' P1-N1-P2 and T-complex split epoch waveforms between 50 and 600 ms.

Word type	FC 4	FC	PT 24	PT
/pet/				
Pearson <i>r</i>	.87	.88	.54	.59
Range	.23 to .97	.51 to .97	-.25 to .92	-.13 to .94
Percentage	98	100	81	92
/pt/				
Pearson <i>r</i>	.83	.84	.5	.5
Range	.09 to .98	.00 to .98	-.58 to .96	-.50 to .93
Percentage	96	96	81	92
/set/				
Pearson <i>r</i>	.84	.86	.53	.6
Range	.03 to .98	.60 to .98	-.26 to .91	-.50 to .92
Percentage	98	100	85	85
/st/				
Pearson <i>r</i>	.85	.85	.45	.56
Range	.58 to .96	.47 to .98	-.45 to .92	-.33 to .94
Percentage	100	100	81	81

Note. Values are shown for each word type at fronto-central (FC) site 4 (FCz), averaged FC sites, left posterior temporal (PT) site 24 (T7), and at averaged left and right PT sites. Percentage of subjects showing significant positive association between the split epoch averages for each word type, at each site analyzed, is listed below these values.

group 1 are shown on the left, and sample individual subject waveform patterns from group 2 are shown on the right. The morphology of the grand mean waveform is evident in individual waveform patterns, although split epoch waveforms are not identical. This suggests that although highly similar, using approximately 70 trials is not sufficient to eliminate all variations. For example, the overlay of split epoch graphs to the /st/ onset for S11 (see Figure 1, bottom left) illustrates that averaging amplitude values of approximately 140 trials would better reflect the P1-N1-P2 grand mean pattern (see Figure 1, top center) to natural speech in some subjects.

Stability of the T-Complex to Multiple Productions of Natural Speech

The T-complex grand mean split epochs to each word type were found to be highly similar (group 1: /pæt/ *r* = .94, /sæt/ *r* = .95, /pt/ *r* = .91, /st/ *r* = .93; group 2: /pæt/ *r* = .95, /sæt/ *r* = .89, /pt/ *r* = .98, /st/ *r* = .96), which demonstrates good representation of sensory processing within individual subject waveforms. Also, a similar pattern of deflections in the grand mean T-complex for groups 1 and 2 (see Figure 2, center) provides additional evidence that the T-complex waveforms of individual subjects contain reliable information pertaining to acoustic and phonological processing.

The mean Pearson *r* values demonstrating the association between T-complex split epoch waveforms for each word type from all 48 subjects within Table 2 show moderate to high positive association, from .45 to .60 for individual subjects. Associations were higher from averaged left and right PT sites than left PT site 24 (near T7) alone,

suggesting bilateral processing of the speech stimuli. Examination of the range of association values for T-complex split epochs, as well as percentage of individuals showing significant associations between T-complex split epochs, demonstrates more variability for T-complex responses than for the P1-N1-P2 responses. Table 2 also shows that greater than 80% of individuals showed a significant positive association between T-complex split epoch patterns. Table 3 (right) illustrates that all subjects showed high positive association, *r* > .50, between split epoch T-complex waveforms for at least one word type, and all except four subjects (i.e., 92% of the subjects) showed high positive association for at least two word types (*r* < .50, highlighted in bold).

Figure 2 provides graphs of split epoch T-complex waveform responses in individual subjects to the /st/ onsets at PT sites that surround the grand mean T-complex graphs (center; also to the /st/ onsets). Individuals' T-complex waveform patterns from group 1 are shown on the left, and individuals' T-complex patterns from group 2 are shown on the right. Individual subjects' split epoch waveforms that illustrate a range of high positive associations are shown. While grand mean patterns are evident in these waveforms, averaging approximately 70 trials was not sufficient to eliminate noise in single-subject waveforms. For example, the T-complex split epoch waveform for subject 26 (see Figure 2, right, top) reflects the grand mean pattern for group 2 (see Figure 2, center, bottom) in only one split epoch waveform (red). Doubling the single-trial responses by averaging responses included in both split epochs (red and blue patterns) at each time point will eliminate variations in split epoch graphs and better reflect the grand mean pattern. Averaging a greater number of trials is clearly necessary for T-complex waveforms, with the exception of only a minority of subjects (see S01, Figure 2, left, top).

Discussion

We have demonstrated benchmarks for stability of cortical sensory P1-N1-P2 and T-complex waveform responses to multiple productions of natural speech that can be used for future studies examining individuals and groups with auditory processing deficits and for studies investigating neuroplasticity following behavioral training. The P1-N1-P2 split epoch averages for responses to naturally produced words showed high positive association at the group and single-subject levels, and the T-complex split epoch averages showed high positive association at the group level. High positive association between split epoch averages suggests good representation of the signal within the AEP waveform (Handy, 2005), which supports our view that the P1-N1-P2 complex and the T-complex, at least at group level, can be used to assess deficits in auditory processing.

Stability of the P1-N1-P2 to Multiple Productions of Natural Speech

The P1-N1-P2 responses to multiple productions of natural speech were highly stable at the group and

Table 3. Pearson *r* values for individual subjects at averaged fronto-central (FC) sites (left) and *r* values at left PT site 24 (T7) and averaged left and right averaged posterior temporal (PT) sites (right) for each word type.

Subject	/pet/	/set/	/pt/	/st/	/pet/		/set/		/pt/		/st/	
	FC	FC	FC	FC	24	PT	24	PT	24	PT	24	PT
S01	.94	.95	.96	.95	.83	.91	.75	.84	.71	.87	.74	.94
S02	.96	.93	.90	.83	.14	.74	.48	.84	.52	.87	.33	.64
S03	.89	.86	.92	.70	.66	.29	.67	.15	.73	.53	-.42	-.33
S04	.96	.98	.97	.95	.65	.70	.65	.77	.46	.48	.32	.72
S05	.89	.90	.83	.89	.90	.75	.80	.77	.88	.67	.55	.74
S06	.93	.72	.85	.80	.80	.88	.69	.83	.58	.68	.86	.89
S07	.93	.94	.89	.89	.45	.54	-.12	.32	.57	.70	.37	.40
S08	.89	.92	.86	.82	.56	.70	.49	.74	.27	.48	.22	.25
S09	.71	.72	.43	.59	.64	.73	.67	.92	.73	.77	.37	.78
S10	.59	.65	.12	.61	.36	.16	.32	.51	.29	.52	.30	.38
S11	.77	.66	.72	.77	.12	.02	.37	.57	-.16	-.04	.24	.41
S12	.89	.87	.93	.89	.30	.29	.81	.54	.68	.61	.44	.23
S13	.90	.90	.87	.76	.05	.35	-.01	.54	-.16	.18	-.17	.25
S14	.86	.71	.00	.60	.46	.69	.61	.76	.11	.38	.43	.48
S15	.97	.92	.91	.98	.75	.81	.67	.46	.69	.88	.39	.39
S16	.78	.81	.64	.73	.42	.52	.42	.43	.48	.62	.55	.77
S17	.96	.93	.88	.90	.86	.94	.74	.73	.71	.79	.57	.74
S18	.60	.60	.54	.80	.26	.41	.27	-.50	-.58	-.50	.50	.57
S19	.77	.88	.86	.84	.59	.42	.83	.70	.77	.56	.58	.30
S20	.82	.83	.82	.85	.80	.06	.51	.21	.18	.34	.66	.33
S21	.51	.72	.81	.80	.67	.76	.66	.74	.24	.53	.68	.93
S22	.92	.86	.92	.89	.48	.61	.34	.27	.37	.43	.53	.40
S23	.79	.70	.80	.83	.88	.80	.60	.81	.72	.74	.72	.80
S24	.68	.75	.86	.47	.60	.87	.56	.81	.79	.80	.24	.24
S25	.94	.91	.95	.96	.10	-.13	.11	.70	.62	.63	.36	.17
S26	.93	.97	.93	.97	.53	.50	.83	.74	.78	.87	.73	.73
S27	.81	.86	.85	.91	.70	.47	.63	.64	.59	.85	.13	.52
S28	.95	.89	.88	.90	.88	.81	.88	.86	.94	.89	.92	.85
S29	.94	.92	.89	.86	.65	.59	.46	.80	.73	.69	.77	.85
S30	.88	.89	.92	.94	.67	.60	.77	.58	.66	.80	.83	.70
S31	.94	.95	.83	.94	.70	.79	-.26	.08	.29	.69	-.11	.67
S32	.92	.96	.98	.93	.32	.29	.05	.50	.48	.27	.31	-.02
S33	.93	.91	.91	.94	.85	.90	.46	.78	.23	.31	.51	.76
S34	.94	.91	.94	.92	.92	.90	.81	.83	.76	.79	.31	.83
S35	.95	.88	.98	.92	.82	.86	.90	.91	.96	.93	.88	.89
S36	.87	.91	.87	.74	.11	.77	.01	.58	-.02	.79	.41	.67
S37	.92	.91	.95	.92	.08	.31	.66	.42	.50	.22	.82	.63
S38	.96	.90	.83	.94	.67	.49	.43	.68	.62	.88	.72	.81
S39	.97	.96	.95	.93	.91	.92	.91	.91	.87	.84	.80	.89
S40	.97	.96	.92	.95	.71	.73	.82	.90	.50	.52	.81	.78
S41	.93	.92	.97	.85	-.14	.80	.83	.87	.88	.73	.80	.84
S42	.95	.96	.92	.94	-.25	.63	.23	.65	.64	.48	-.39	.17
S43	.83	.63	.74	.83	.40	.63	.43	.43	.38	.32	.57	.70
S44	.94	.97	.93	.79	.74	.68	.32	.81	.88	.81	-.45	.15
S45	.97	.93	.97	.94	.39	.39	.56	.13	.72	.54	.55	.59
S46	.96	.76	.95	.94	.55	.58	.29	.33	.32	.61	.30	.58
S47	.97	.94	.97	.93	.55	.58	.78	.66	.52	.60	.49	.56
S48	.93	.91	.92	.94	.63	.40	.56	.16	-.20	-.11	.41	.31

Note. Values that fail to show a high positive association (i.e., $r < .50$) are shown in boldface. Supplemental Material shows Pearson *r* values for individuals at FC site 4 (near Fz) for each word type. All other values are shown here.

single-subject levels, with all subjects showing high positive association between split epochs for at least three word types from averaged FC sites. The associations for P1-N1-P2 split epochs at the single-subject level were also highly associated for a single FC electrode site (site 4, near Fz), with 47 out of 48 subjects showing high positive split epoch associations for three word types. Sensory waveforms obtained at the single FC electrode site and those averaged from four FC electrode sites were similar; however,

averaging amplitudes obtained at four FC electrodes sites that reflect sensory processing further eliminated noise within the signal. This result suggests that future research comparing stability of synchronous neural responses to natural speech in language learners with typical and atypical development should compare whether analysis of the FC cluster of electrodes used in the current study in individual subjects or a more extensive cluster of FC electrodes obtained through identification of electrode

Figure 2. The grand mean T-complex split epoch waveform responses to the /st/ onset at averaged posterior temporal (PT) sites (center). Sample split epoch T-complex waveform responses to the /st/ onset at PT sites from individual subjects surround the grand mean T-complex waveforms. Group 1 sample subject waveforms are shown on the left, and group 2 sample subject waveforms are shown on the right. Waveforms between 50 and 600 ms are shown, which correspond to the time interval used to obtain the Pearson r values shown. Scales for individual subjects vary to best illustrate waveform morphology. Note that group 2 data include additional data points, therefore, colors appear darker. Red = split epoch, half of trial responses to /st/ onset; blue = split epoch, remaining half of single trial responses to /st/ onset.

data clusters (Maris & Oostenveld, 2007) provides measures of sensitivity and specificity appropriate for diagnosis of auditory processing deficits.

The grand mean P1-N1-P2 split epochs to /pæt/, /sæt/, /pt/, and /st/ were replicated in two independent subject groups using approximately 70 single trials per subject. High similarity of the grand mean split epochs in the two subject groups suggests good signal representation relative to noise within the individuals' responses. At the single-subject level, associations between the P1-N1-P2 split epochs are high; however, Pearson r values in Table 2 and sample graphs in Figure 1 demonstrate that averaging more than 70 single trials would be beneficial to further eliminate noise from the signal waveform.

Sensory responses at FC electrode sites to nonspeech stimuli (Pekkonen, Rinne, & Näätänen, 1995) and single productions of natural speech have been shown to have good

intrasubject stability (Tremblay et al., 2003). In this study, we extended these findings to reveal excellent intrasubject stability of P1-N1-P2 responses to speech that varies in a naturalistic manner from a single speaker using approximately 70 single trials. Hence, these findings support our view that the P1-N1-P2 responses to natural speech can be used to assess recognition of spectro-temporal feature changes within the phoneme sequence of the spoken word and to assess stability of synchronous neural responses to natural speech at cortical levels in individuals with auditory processing deficits associated with language impairment. The current results provide the necessary benchmarks for stability of neural responses to natural speech in individual adult subjects. It will be necessary in future studies to also establish typical patterns of stability of responses in children and adolescents.

The current results also provide baseline measures for future studies examining the cortical sensory responses

following behavioral training in individual subjects. Tremblay, Ross, Inoue, McClannahan, and Collet (2014) investigated behavioral and AEP responses in three groups of 10 subjects who had different amounts and types of exposure to single productions of the syllables “mba” (representing prevoicing for the syllable “ba”) and “ba.” Increases in the P2 amplitude were related to levels of exposure to the stimuli but not to improvements in perception through active training. The researchers speculated that the changes in P2 might reflect a precursor to learning. Although not discussed by the authors, it is also possible that the demonstrated changes in the P2 responses in the adult subjects reflected increased attention to language stimuli that may facilitate perception. Future studies might assess whether attention and/or learning increases phase-locked responses, and therefore the stability of responses to the auditory stimulus reflected within the P2 response. Benchmarks provided in this study will allow researchers to evaluate variation in sensory AEP responses as valid effects of manipulated variables such as learning, motivation, or attention.

Stability of the T-Complex to Multiple Productions of Natural Speech

The T-complex split epoch averages for responses to each word type using approximately 70 responses were highly reliable at the group level. The highly similar T-complex waveform patterns found for each word type for two independent groups of adults suggest that the T-complex contains useful information pertaining to acoustic and phonological processing (Chang et al., 2010; Mesgarani et al., 2014; Wagner et al., 2013) and is sufficiently reliable, at the group level, to assess the stability of neural responses in populations with auditory processing deficits.

At the single-subject level, we found inter- and intra-subject variability. The T-complex showed positive associations between split epochs in greater than 80% of the subjects and showed high positive associations ($> .50$) for at least one word type for all 48 subjects and high positive associations for two word types for 92% of the subjects. High intersubject variability in T-complex responses has been previously reported (Bishop et al., 2012; Shafer et al., 2011). By examining intrasubject variability across four word types, our results revealed that single-subject T-complex waveform patterns that differ from the grand mean T-complex pattern are not specific to particular subjects. For example, only 11 out of 48 subjects showed high positive association to all four of the word types. Thus, it was not unusual for subjects to show a high positive association for one word type but not other word types (see Table 3, subjects S15, S25, and S43). Also, some subjects showed high positive association for waveforms at the left PT site 4 (T7) but not at the averaged PT sites (see Table 3, subjects S3 and S20), which also suggests intrasubject variability, because averaging responses from left and right PT electrode sites showed further reduction of noise for most subjects. T-complex variability may result from cellular or sensory fluctuations or induced non-phase-locked neural activity

(Faisal et al., 2008; Gilley & Sharma, 2010). Because the T-complex reflects activity within a focal region within the posterior lateral superior temporal gyrus (Howard et al., 2000), we speculated that electrode placement due to variation in head shapes and sizes could result in intrasubject variability. The current results, which found high positive associations for at least two word types in most subjects, suggest that intrasubject variability for most subjects cannot only be explained by electrode placement or variation in head shapes and sizes. The superior temporal gyrus has multiple neighboring neural ensembles that differ in function (Boatman, 2004), and focal regions associated with acoustic-phonetic and linguistic processing within the posterolateral superior temporal gyrus vary from individual to individual (Nourski, Steinschneider, Oya, Kawasaki, & Howard, 2015; Steinschneider et al., 2014), which may explain T-complex variability in individual subjects.

Our results suggest that the T-complex as a diagnostic measure of stability of neural responses at cortical levels in individual subjects lacks specificity. However, because all 48 subjects showed high positive association ($> .50$) to at least one word type, and all except four subjects showed high positive association to at least two out of the four word types, the T-complex may be used to rule out instability of neural responses at the single-subject level. Furthermore, good signal within the individual subject's T-complex even to one word type allows for comparisons between acoustic-level processing reflected within the P1-N1-P2 complex and acoustic and phonological levels of processing reflected within the T-complex at the single-subject level.

Variability in the Cortical Sensory Waveforms of the AEP

The current results add an additional measure for assessing variability of AEP cortical sensory waveforms. In an earlier study (Wagner et al., 2016), we questioned whether variability of the P1-N1-P2 complex and T-complex responses to the phoneme sequence onsets /pət/ and /sət/ would remain stable or increase in variability as time increased between 50 and 600 ms. We reasoned that variability in single-trial responses would increase as time increased because with increasing time from word onset, additional neural sources contribute to the AEP response (Steinschneider et al., 2011). Stability was assessed using the standard deviation of individual subjects' P1-N1-P2 and T-complex amplitude values at each data point between 50 and 600 ms. Our prediction was not supported in that we found that variability for the P1-N1-P2 complex and T-complex responses remained stable or decreased with increasing time through 600 ms from stimulus onset. Thus, nonsense word forms may be best for examining acoustic-phonetic and phonological aspects of processing because processing is stable, as measured by standard deviation, through 600 ms. A future study comparing real and nonsense words will be needed to test this claim.

We have demonstrated that P1-N1-P2 complex and T-complex waveform morphology is highly similar in English

and Polish subjects, even for the /pt/ phoneme sequence onset, which occurs only in the Polish language (Wagner et al., 2013, 2016). Thus, in the current study, grand mean P1-N1-P2 complex and T-complex waveforms included split epoch responses from individuals in both language groups. Statistical analysis used to compare language group responses in our previous studies accounted for variability in P1-N1-P2 and T-complex amplitudes. Perception, however, is facilitated in the auditory cortex at multiple stages and levels of neural processing (Gilley, Sharma, & Purdy, 2016; Nourski et al., 2015); thus, additional investigations will be necessary to understand the manner in which acoustic-level processing at early cortical stages is enhanced to form native-language patterns of perception in native English and native Polish listeners. For example, in our laboratory, we are currently manipulating the effects of attention on the cortical sensory responses in English and Polish listeners to further explore this question.

Conclusion

This study provided benchmarks for stability of neural activity reflected within the P1-N1-P2 complex and T-complex of the AEPs. The P1-N1-P2 complex responses are sufficiently reliable at both the group and single-subject levels to use for comparison in assessments of stability of neural responses in individuals with auditory processing deficits associated with language impairment, dyslexia, and autism. The T-complex responses are sufficiently reliable at the group level to assess stability of neural responses in populations with auditory processing deficits. Also, results found that all subjects showed highly associated T-complex split epoch waveforms to one out of four word types, and 92% of subjects showed highly associated patterns to two out of four word types. Thus, the T-complex at the single-subject level may be useful for comparing waveforms that support acoustic or acoustic and phonological levels of processing. Hence, T-complex responses in individual subjects can rule out instability of responses in individuals with auditory processing deficits and identify level of deficit, specifically, whether the origin is in the superior temporal plane or lateral posterior superior temporal gyrus. This information is important because the cortical sensory waveforms have been found to reflect recognition of the spectro-temporal features of the spoken word (Wagner et al., 2016). Each phoneme sequence has a distinct series of acoustic feature changes that affect the waveform morphology in a unique manner, thus revealing cortical level recognition of the phoneme sequence within the spoken word.

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