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Investigating infant auditory processing and event-related brain oscillations
--Manuscript Draft--

Abstract:	Rapid auditory processing and acoustic change detection abilities play a critical role in allowing human infants to efficiently process the fine spectral and temporal changes that are characteristic of human language. These abilities lay the foundation for effective language acquisition; allowing infants to hone in on the sounds of their native language. Invasive procedures in animals and scalp-recorded potentials from human adults suggest that simultaneous, rhythmic activity (oscillations) between and within brain regions are fundamental to sensory development; determining the resolution with which incoming stimuli are parsed. At this time, little is known about oscillatory dynamics in human infant development. However, animal neurophysiology and adult EEG data provide the basis for a strong hypothesis that rapid auditory processing in infants is mediated by oscillatory synchrony in discrete frequency bands. In order to investigate this, 128-channel, high-density EEG responses of 4-month old infants to frequency change in tone pairs, presented in two rate conditions (Rapid: 70 ms ISI and Control: 300 ms ISI) were examined. To determine the frequency band and magnitude of activity, auditory evoked response averages were first co-registered with age-appropriate brain templates. Next, the principal components of the response were identified and localized using a two-dipole model of brain activity. Single-trial analysis of oscillatory power showed a robust index of frequency change processing in bursts of Theta band (3-8 Hz) activity in both right and left auditory cortices, with left activation more prominent in the Rapid condition. These methods have produced data that are not only some of the first reported evoked oscillations analyses in infants, but are also,

	importantly, the product of a well-established method of recording and analyzing clean, meticulously collected, infant EEG and ERPs. In this article, we describe our method for infant EEG net application, recording, dynamic brain response analysis, and representative results.
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1 **TITLE:**

2 Infant auditory processing and event-related brain oscillations

3

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44 **KEYWORDS:**

45 Infant; Infant Brain; Human Development; Auditory Development; Oscillations; Brain
46 Oscillations; Theta; Electroencephalogram; Child Development; Event-related Potentials; Source
47 Localization; Auditory Cortex
48
49 **SHORT ABSTRACT:**
50 High-density electroencephalography (dEEG) is being used increasingly to study brain
51 development and plasticity in the early years of life. Here we present an application of
52 sophisticated analysis techniques that builds on traditional EEG recording to understand the
53 oscillatory dynamics of rapid auditory processing in the infant brain.
54
55 **LONG ABSTRACT:**
56 Rapid auditory processing and acoustic change detection abilities play a critical role in allowing
57 human infants to efficiently process the fine spectral and temporal changes that are
58 characteristic of human language. These abilities lay the foundation for effective language
59 acquisition; allowing infants to hone in on the sounds of their native language. Invasive
60 procedures in animals and scalp-recorded potentials from human adults suggest that
61 simultaneous, rhythmic activity (oscillations) between and within brain regions are fundamental
62 to sensory development; determining the resolution with which incoming stimuli are parsed. At
63 this time, little is known about oscillatory dynamics in human infant development. However,
64 animal neurophysiology and adult EEG data provide the basis for a strong hypothesis that rapid
65 auditory processing in infants is mediated by oscillatory synchrony in discrete frequency bands.
66 In order to investigate this, 128-channel, high-density EEG responses of 4-month old infants to
67 frequency change in tone pairs, presented in two rate conditions (Rapid: 70 ms ISI and Control:
68 300 ms ISI) were examined. To determine the frequency band and magnitude of activity,
69 auditory evoked response averages were first co-registered with age-appropriate brain
70 templates. Next, the principal components of the response were identified and localized using a
71 two-dipole model of brain activity. Single-trial analysis of oscillatory power showed a robust
72 index of frequency change processing in bursts of Theta band (3-8 Hz) activity in both right and
73 left auditory cortices, with left activation more prominent in the Rapid condition. These
74 methods have produced data that are not only some of the first reported evoked oscillations
75 analyses in infants, but are also, importantly, the product of a well-established method of
76 recording and analyzing clean, meticulously collected, infant EEG and ERPs. In this article, we
77 describe our method for infant EEG net application, recording, dynamic brain response analysis,
78 and representative results.
79
80 **INTRODUCTION:**
81 Across a wide spectrum of developmental disorders, it is becoming increasingly clear that the
82 key to early identification and ultimately remediation lies in understanding the early
83 mechanisms that come into play as the developing brain assembles functional networks. Thus,
84 there is increased interest in understanding the temporal dynamics of neural patterns that
85 impact cognition. In particular, specific cognitive functions to be differentially correlated with
86 oscillatory activity in specific frequency bands (e.g. cyclic fluctuations single-cell or population
87 membrane potentials) [1]. Previous studies have established that oscillatory dynamics play a
88 crucial role in the activity-dependent self-organization of developing networks [2-4], control

89   neuronal excitability [5,6] and integrate sensory inputs [7,8]. Oscillatory brain activity is thought to
90   be metabolically beneficial [9,10], increasing the efficiency of a variety of sensory processing
91   functions and coordination of higher-level functions such as cognition and language. However,
92   systematic investigation of the role of neural synchrony across age and links with behavioral
93   outcomes in human infants has yet to be accomplished. An important step toward this
94   objective is to achieve a deeper understanding of the emergence and maturation of the
95   temporal dynamics and oscillatory mechanisms that support developing cognitive processes
96   including early language.
97
98   A crucial component of language development is the ability to accurately process and
99   categorize acoustic signals that change rapidly: often on the order of as little as *tens of*
100 *milliseconds*. For example, the acoustic dynamics of the words "dad" and "bad" differ
101 acoustically only over the first 40 ms of the syllable, yet the two have very different meanings
102 and associations. Previous studies show a maturational trajectory of receptive ability for
103 acoustic and linguistic differences. As early as 2 months of age, infants show the ability to
104 discriminate rapid frequency changes (e.g. <100 ms); suggesting that the "hardware" for
105 detecting the difference between two acoustically similar syllables is in place. Over the next few
106 months, babies can discriminate increasingly smaller differences, develop categorical
107 perception, and exhibit cortical specialization for sounds of the native language syllables [11-14].
108 Because complex sound perception relies on the function of basic processing mechanisms, it is
109 thought that deficits in the ability to perceive rapidly changing acoustic differences – even for
110 simple sounds such as tones – may be early indicators of [15] later language impairment.
111
112 Previous work from Choudhury and Benasich in this laboratory strongly supports this
113 hypothesis, showing that an infant's ability to process very rapid changes in simple sounds (e.g.
114 tones) can predict 3- and 4-year language and cognitive abilities [16,17]. These data verify that the
115 brain responses of pre-lingual infants can provide a quantifiable indicator of auditory
116 processing and developmental progress. The study and methods presented here probe key
117 aspects of the underlying mechanism of this relationship. Several lines of research now indicate
118 that peak latency and amplitude features of ERP features arise from the summation of
119 spectrotemporal dynamics in EEG oscillations of multiple generators [18-23]. Spectrotemporal
120 analysis also allows the separation of phase and power information. Phase-locked activity
121 reflects the part of the neuronal response that is evoked by the stimulus. This type of
122 information is similar to what can be extracted from the ERP, since responses are averaged
123 relative to a time-locked event. However, the timing of some neuronal activity may vary from
124 trial to trial. In ERP analysis, this activity is "averaged out"; however in analysis of power
125 changes from trial to trial, this information can be recovered and analyzed. Therefore,
126 spectrotemporal analysis of phase and power may give additional information about the
127 neuronal response, relative to the conventional ERP. Regarding infant development, there is
128 considerable evidence that oscillations contribute to the development of neural circuits in
129 animal models [2,3] but these mechanisms are only beginning to be investigated in the human
130 population. Work from this laboratory has shown theta and gamma oscillatory correlates of
131 native language specialization at 6-months [24]. This highlights the functionality of oscillatory
132 hierarchies in infancy.

133

134 The global hypothesis, based on the evidence presented above, is that synchrony of evoked
135 oscillations in auditory cortices supports infant brain development. As a first step in testing this
136 hypothesis, a "baseline" of processing in early infancy was obtained; namely, 4-months-of-age,
137 which is currently thought to precede "perceptual narrowing" for native language specialization
138 [25,26]. Accordingly, we performed single-trial frequency analysis on infant EEG data recorded
139 during passive listening to pitch-variant and pitch-invariant tone pairs presented in an "oddball
140 paradigm" consisting of two rate conditions (Control condition: 300 ms inter-stimulus interval;
141 Rapid condition: 70 ms inter-stimulus-interval).

142

143 Here we illustrate this method using stimuli from studies focusing on rapid auditory processing.
144 In these studies, an "oddball paradigm", was used to assess neuronal activity to unpredictable,
145 but recognizable events. In this paradigm, the brain response to unpredictable or "odd" stimuli
146 are often called "Deviant" responses, whereas the response for the predictable stimulus,
147 presented most of the time, is usually called the "Standard" brain response. Responses to
148 stimuli presented in an oddball paradigm can be automatically elicited without focused
149 attention, making this paradigm easy to use with very young infants. All of the auditory stimuli
150 are presented via free-field speakers at intervals, which vary depending on the study. As
151 mentioned previously, in the current study sounds that index rapid auditory processing (RAP)
152 abilities were used: that is, sounds containing tens-of-milliseconds of acoustic change [16,17,27,28].
153 It may be noted that many other stimulus types are useful for testing neurophysiological
154 discrimination, including consonant-vowel (CV) sounds as well as deviants reflecting changes in
155 Frequency or Duration, with an interposed Gap, and/or ascending or descending frequency
156 Sweeps. Finally, we also recommend recording spontaneous EEG during "quiet play" in which
157 no auditory stimulus is presented. These data may then be used to measure oscillatory coupling
158 and coherence in the absence of repeated stimulation.

159

160 Recording EEG activity from an infant population poses a set of unique challenges. For example,
161 cooperation with placement of the electrodes and leaving them in place for the duration of the
162 experiment, minimizing movement to prevent EEG artifacts, and keeping the baby engaged and
163 distracted with silent toys all represent challenges. Additionally, infant data do not easily lend
164 themselves to straightforward applications of protocols developed with adult/older child data.
165 In many instances the relationship between components observed in infant EEG and event-
166 related potentials (ERPs) is not as clear cut nor does it always map on to what is accepted in the
167 adult. While developmental research holds a powerful potential for understanding the genesis
168 of typical and disordered brain function, recording reliable and interpretable brain responses
169 from human infants requires a high level of proficiency in both technical and interpersonal
170 realms. These challenges, however, can be overcome and reliable EEG and ERP data can be
171 recorded from infants of different ages using a variety of paradigms. Here we describe a general
172 method of analysis utilizing commercially available ERP recording and analysis software in
173 combination with a free, open-source ERP analysis package that works in the MATLAB
174 environment [29].

175

176 The application of oscillatory analysis methods to infant brain response recordings allows

177 exploration of more mechanistic questions of neuronal synchrony development in relation to
178 language acquisition and putative underlying mechanisms when that synchrony is
179 compromised. Related efforts using other stimuli, such as speech syllables [24], and analysis of
180 spontaneous or "resting" oscillations [1] in longitudinal analyses or in combination with early
181 training paradigms, offer windows into the temporal, spatial, and spectral dynamics of typical
182 and disordered developmental trajectories. It is hoped that these efforts will increase our
183 understanding of the bases of auditory development and plasticity, and aid in identification and
184 remediation strategies for developmental language disorders.
185
186 **PROTOCOL:**
187
188 All work with human subjects requires Institutional Review Board approval and oversight.
189 Methods reported here, when used in research, have been reviewed and approved by the
190 Human Subjects Protection Program through the Rutgers Arts and Sciences Institutional Review
191 Board (IRB).
192
193 **1. Preparation**
194
195 1.1.   Schedule the baby for 2 hours of testing in a sound-attenuated and electrically-shielded
196 chamber. The study protocol presented in this article that produced representative data
197 includes 20-30 minutes of EEG testing.
198
199 1.2.   Allocate three people per testing session: one "primary tester", one "net assistant" and
200 one "entertainer".
201
202 1.3.   For high-density infant EEG recording, use at least a 64-channel net. For the
203 representative data presented here, a 128-channel sensor net was used.
204
205 **2.   Net Application**
206
207 2.1.   Set up the following supplies in the recording chamber: Coban self-adherent wrap tape,
208 measuring tape, marking pen, 2 towels, and pipettes. Calibrate sounds to testing level (e.g. dB
209 SPL, HL, etc.).
210
211 2.2.   Make the electrolyte solution (distilled water, Potassium Chloride and baby shampoo)
212 well before estimated family arrival time. To ensure that the net is not too cold on the baby's
213 head, warm 8 ounces of water to be added to the solution just before application.
214
215 2.3.   When the family arrives, obtain consent with IRB approved forms.
216
217 2.4.   In the testing chamber, sit the infant on the caregiver's lap, and have the entertainer
218 begin to play with the infant. If it is the first visit, explain the net application procedure.
219
220 2.5.   Measure the infant's head circumference at the widest point of the head and choose

221 the net size based on this measurement. To obtain the best fit, choose a smaller-sized net if the
222 circumference is close to the minimum size for one age. Submerge the chosen net in the
223 electrolyte solution.
224
225 2.6.   Measure nasion-to-inion and mark the scalp at ½ of the total measurement. Do the
226 same for the ear-to-ear measurement. The final mark is Cz (vertex).
227
228 2.7.   Remove excess solution from the net by placing it on a dry towel. Have the net assistant
229 invert the net and grasp the Cz electrode; holding the net so that the primary tester can
230 position their fingers at the front of the net.
231
232 2.8.   Flip the net over and place the net on the infant head as the net assistant moves the
233 chinstrap and colored front threads (connecting the nasion electrode and chinstrap) outside of
234 the net.
235
236 2.9.   Adjust the net position on the infant's head, placing Cz at the vertex scalp mark.
237 Position/align each of the electrodes starting from the back and working toward the front,
238 making sure that there is a right angle between each electrode and the head surface.
239
240 2.10.   Collect the wires, adjust the chinstrap, secure wires with Coban tape, and plug in the net
241 connector.
242
243 2.11.   Measure the electrode impedance with a threshold of <50 KOhms, or according to
244 system instructions. If some electrodes have high impedance, re-soak the electrode with an
245 electrolyte-filled pipette and gently move the hair from beneath the electrode.
246
247 **3.   Stimuli Presentation and EEG Recording**
248
249 3.1.   Present auditory stimuli in free-field with speakers equidistant from the infant's head.
250
251 **NOTE:** The representative study stimulus parameters were as follows: 70 ms tone-pairs with a
252 fundamental frequency of either 800 or 1200 Hz and 15 harmonics (6 dB roll-off per octave) are
253 presented in two blocks (70 or 300 ms inter-stimulus interval). The low-high pairs (800-1200 Hz)
254 are presented as deviants (15 % = 125 trials) among low-low (800-800 Hz) standards (85% = 708
255 trials).
256
257 3.2.   Record the EEG according to hardware and software instructions. Use the following
258 parameters for the representative data: Sampling rate: 250 Hz, Low-pass Hardware filter: 100
259 Hz, Elliptical, High-pass filter: 0.1 Hz, Auto: set to Nyquist, Board gain: 1.
260
261 3.3.   Provide a calm, quiet, mildly engaging environment for the infant during recording.
262 Engage the infant by playing an age-appropriate silent video or with soft toys (e.g. blow
263 bubbles, point to pictures in books, puppet play). Providing the caregiver with earphones to
264 listen to music avoids inadvertent caregiver interference with the infant response. If the infant

265 is restless, impacting the EEG, pause stimulus presentation and EEG recording until a calm
266 environment can be restored.

268 3.4. After the experiment is completed, gently remove the net and dry the infant's hair and
269 head.

271 3.5. Save and backup the raw, unfiltered EEG data before closing the programs at the end of
272 the testing session.

274 **4. Data Processing - ERPs**

276 4.1. Visually inspect the raw EEG data and reject segments with high-amplitude artifact.

278 **NOTE:** Reject channels with high amplitude and interpolate. Maximum percent of rejected
279 channels should be set at 30%. Alternative methods (e.g. ICA, PCA) [30] may be employed to
280 reduce or reject artifacts present in the data.

282 4.2. Filter the data with pre-established parameters that accord with cortical activity. For
283 infants, use an off-line bandpass filter of 1-15 Hz.

285 4.3. Segment the continuous data to create epochs around "Time 0" (stimulus onset)
286 according to software instructions. For segmentation, include sufficient pre-stimulus time to
287 establish baseline activity and post-stimulus time to capture the entire response.

289 4.4. Reject noisy epochs according to an appropriate rejection criteria (e.g. +/- 200 µV for
290 infants). Set maximum percent of rejected epochs set at 30% for inclusion.

292 4.5. Average the epochs for each individual and each condition and combine these averages
293 according to group and condition for grand averages.

295 4.6. If the number of epochs contained within each average varies across subjects, weight
296 the number of epochs so that subjects with more/less epochs are equally valued.

298 4.6.1 To weight the grand average, calculate the individual average from each condition as n
299 times the waveform, where n equals the number of epochs that comprise the average, then
300 divided by the total number of epochs for all subjects. This method gives each trial equal weight
301 in the final average.

303 **5. Data Processing – Source Localization**

305 5.1. For infant data, co-register each individual and grand average ERP file with either an
306 age-appropriate MR template or an individual MR scan (refer to previous publications [31,32]).

308 **NOTE:** In the co-registration process, the electrode positions and reconstructed head are

309 registered into a single coordinate system. Grand averages may be used to define the dipole
310 model.
311
312 5.2.   Evaluate the scalp thickness, skull thickness, width of the subarachnoid space and bone
313 conductivity must be accurately and enter the age group as these factors change rapidly during
314 development. For the representative data, parameters are: skull: 1.5 mm; scalp: 2.5 mm;
315 subarachnoid space: 1.7 mm; bone conductivity: 0.0581.
316
317 5.3.   Beginning with a grand average ERP, choose a time window of interest corresponding to
318 a peak. Conservative parameters are typically +/- 20 ms around the peak of interest [31].
319
320 5.4.   Estimate the number and location of underlying sources to be fitted to the data. For an
321 auditory paradigm, use two dipoles with free location and rotation.
322
323 **NOTE:** Source estimation is then automatically guided through a minimization of a cost function
324 that is a weighted combination of 4 residual fit criteria to obtain the "best fit" location to the
325 time window of interest.
326
327 5.5.   Check the "goodness of fit" for the dipole solution using the software outputs of
328 residual variance. This is the amount of signal that remains unexplained on the given time
329 window of fit by the current dipole model. Adjust the time window to minimize the residual
330 variance. Use a distributed source model (CLARA) to check the solution within the extended
331 activity region.
332
333 5.6.   Save the dipole-source solution and waveforms for each condition and peak.
334
335 5.7.   Repeat the procedure for every individual average file.
336
337 5.8.   For statistical analysis of source location, use values of peak latency, amplitude, and
338 location coordinates in the X (medial-lateral), Y (anterior-posterior), and Z (superior-inferior)
339 directions for each dipole from each individual average file solution. In the case presented here,
340 2X2 Repeated measures ANOVA (Stimulus (standard, deviant) X Hemisphere (Left, Right) for the
341 amplitude and latency of the sources can be useful to look at strength and timing of the
342 generators. Source coordinates can be evaluated in the same way [for review, [31,32]].
343
344 6.   **Data Processing – Time-Frequency Analysis in Source Space**
345
346 6.1.   Apply the dipole model solution to the raw, unfiltered, continuous EEG data.
347
348 6.1.1.   Apply the source solution (saved in step 5.6.) to the raw EEG data file as a virtual
349 electrode montage.
350
351 NOTE: The use of the dipole model in this way applies a fixed spatial filter onto the surface
352 channel recording (sensor space) to transform the continuous high-density EEG into a virtual 2-

353   source montage (brain source space).

354

355   6.2.   Transform the time-domain single-trial source signal into the time-frequency-domain
356   (Figure 1).

357

358   NOTE: Currently, several approaches can be utilized to transform single-trial data into the time-
359   frequency domain, including wavelet analysis and application of a Hilbert transform to filtered
360   data. While a comparison of these methods is outside the scope of this article, several
361   published articles have thoroughly described these methods [33-36]. The time-frequency analysis
362   using the complex demodulation procedure found in a commercially available coherence
363   software program [37], calculates the instantaneous envelope amplitude and phase of each brain
364   activity as a function of frequency and time [37-39]. This produces measures of instantaneous
365   power shifts (Temporal Spectral Evolution, TSE) and phase-locking (Inter-trial Phase Locking,
366   ITPL).

367

368   6.2.1.   Use the following parameters: 1 Hz wide frequency bins from 2 to 80 Hz and 50 ms time
369   resolution from -1500 to 1500 ms. The epoch time window should be long enough to allow
370   filtering or processing at the lowest desired frequency without incurring artifacts [40,41].

371

372   6.3.   Visualize the frequency peaks of activity in EEG in order to avoid interpretation of
373   possible spurious artifact-related or circular related oscillations [41,42].

374

375   6.4.   Perform permutation and testing cluster analysis to determine regions of significant
376   differences across conditions and groups [24,43] .

377

378   *[Place Figure 1 around here]*

379

380   **REPRESENTATIVE RESULTS:**
381   **Infant Event-related Potentials**
382   Infant ERPs are generally larger than adult ERPs, and may have fewer or more peaks of
383   activation, relative to mature responses, depending on the age [44]. Here, we show
384   representative Grand Average responses from twenty three 4-month-old infants [43] (Figure 2).
385   The oddball paradigm allows us to determine whether the infant's brain can recognize the
386   difference between two events. In the representative results, the tone-variant, deviant
387   response (DEV, 800-1200Hz, red line) elicits an additional peak of activation, relative to the
388   invariant tone pairs (STD, 800-800Hz, black line). This finding is apparent in both Control rate
389   (300 ms ISI, left) and Rapid Rate (70 ms ISI, right) conditions. Example responses from
390   electrodes of Fz (Frontal midline), C3 (Central, right) and C4 (Central, left) are shown. The
391   computed difference wave (Deviant minus Standard) is also shown in gray lines. The additional
392   peak of activation suggests that the infant brain at this age can discriminate the difference
393   between the tones at both rate presentations.

394

395   *[Place Figure 2 around here]*

396

Infant source waveforms

Source activity with little residual variance should follow the ERP peaks, signifying a "good fit" between the original data and the source localized transformed data. In the representative data, we show the location of the two-dipole best fit source model of the infant grand average ERP to the STD (tone-invariant) condition over the CLARA distributed model (Figure 3). The computation clearly shows left and right auditory activation in Control and Rapid Rate conditions.

[Place Figure 3 around here]

Peaks of activity from the two-dipole model (Figure 4) corresponded to the ERP response very well. The peak timing and morphology of the ERP waveforms, shown in panel (i), match the timing and morphology of the source waveforms shown in panel (ii) (for more details, see original article, [43]). Source waveforms from this experiment explained 97.9% of the variance in activity over the scalp electrodes. Statistical analysis of the source peak latencies showed that right hemisphere activity was faster than the left in both conditions, and responses in the rapid rate were later in both hemispheres than in the control condition. Hemispheric differences were not observed using the ERP data, suggesting that the source localization techniques enabled the retrieval of additional information from the responses.

[Place Figure 4 around here]

Infant Event-related Oscillations

In general, time-frequency analyses of adult and animal data show that stimuli evoke a 1/f pattern of neuronal synchrony (e.g., decreasing power with increasing frequency). In the representative data, evoked by auditory tone pairs, we show that infants also express this pattern (Figure 5). Here, stimulus onset elicits synchronous bursts of theta (5-6 Hz), beta (20-25) Hz and gamma (35-45 Hz) power in both right and left auditory regions of the brain.

[Place Figure 5 around here]

Animal models and adult experiments suggest that oscillatory synchrony, and in particular low-to mid-frequency oscillations (e.g. 1-8 Hz) are major contributors to evoked potentials [45]. Analysis of instantaneous power shifts (Temporal Spectral Evolution, TSE) in infant oscillations from our previous publication [43] showed greater induced power to the variant tone in the theta band (6-8 Hz), relative to the invariant tone. This effect was observed in both rate conditions, particularly over the right auditory region in the Control rate condition (Figure 6). Rapid rate presentation yielded a more bilaterally symmetrical activity, suggesting enhanced left cortical involvement during auditory processing of rapidly occurring stimuli and in particular during acoustic change processing.

[Place Figure 6 around here]

Figure 1: Steps of time-frequency analysis.

441 Time-frequency analysis method is illustrated using grand average (n=12) data from 4-month-
442 old infants during the 70 ms ISI tone condition. Stimulus onsets are shown in red arrows
443 beneath the time axis. Steps of analysis: 1. Averaged ERPs, shown in Cz electrode, are created
444 for each channel. 2. Source location of ERP generators, shown in a sketch head, is obtained by
445 using a 2-dipole model in data mapped onto an infant MRI template. 3. Individual and grand
446 average source waveforms are obtained from the fit of the Left and Right dipoles. Infant head
447 models show the voltage maps corresponding to the selected peak (in gray). 4. The source
448 montage is applied to the 128 channel scalp data, and amplitudes are computed and saved for
449 the two source channels. 5. Event related oscillations are calculated from single-trials and
450 averaged over the response period.

451

452 **Figure 2: Event-related potential morphology.**
453 Grand Averages (n=23) to Rapid (70 ms ISI) and Control (300 ms ISI) rate responses to standard
454 (STD, black lines) and deviant (DEV, red lines) tone pairs are shown in frontal midline and
455 central left and right electrodes. Negativity is plotted up. Stimulus onsets are shown in red
456 arrows beneath the time axis at Fz. P1 is shown in the Fz panel with a black arrow. The
457 difference wave (response to DEV minus response to STD) is shown in gray lines (Adapted from
458 [43]).

459

460 **Figure 3: Source localization results.**
461 Two-dipole "best fit" source model is shown overlaid on distributed activity from the source
462 model. Clear left and right activity can be seen over left and right temporal lobe regions.
463 (Adapted from [43]).

464

465 **Figure 4: Event-related Potential and Source Waveform Comparison.**
466 i. Example ERPs from frontal left and right electrodes (F3 and F4) show peaks of activation to
467 tone pairs with invariant and variant fundamental frequencies (STD and DEV, respectively). A
468 change in frequency elicits larger peaks ~ 400 ms (DEV, red line), relative to the when
469 frequencies are unchanged (STD, black). ii. The latency of peaks of activation is similar for the
470 source-localized dipole activity, suggesting a good match between ERP and source waveform
471 analysis. The large peak at 400 ms is particularly noticeable in the right hemisphere with the
472 source-localized data. For simplicity, only the responses to the Rapid Rate condition are shown,
473 however a similar match was also observed between ERP and source waveforms for the
474 responses in the Control Rate condition.

475

476 **Figure 5: Pooled TSE maps are expressed in terms of percent spectral change over an epoch of**
477 **-1 to 1 sec of time for left and right generators.**
478 i. Tones in the 300 ms ISI condition elicit event-related oscillations in coherent frequency bands
479 around stimulus onset (e.g. -1140 ms and 0 ms). A long stimulus epoch is used in order to
480 visualize more of the data and to provide a long enough sample for frequency decomposition.
481 Right panel shows the average spectrum over the initial processing peak (150-300 ms). The
482 average spectrum shows an overall 1/f spectrum with discrete peaks of synchrony at specific
483 frequency bands. ii. A similar pattern is observed for the 70 ms ISI condition.

484

Figure 6: Time-frequency analysis of event-related oscillations in 4 month-old infants.
Change in oscillatory power is shown in Temporal Spectral Evolution (TSE) grand average plots for 4-month-old infants in the Control (A) and Rapid Rate (B) conditions. Black bars on the x-axis illustrate tone onset and durations. Left and Right source activity is indicated in the top left corner of each graph. First row: i.Responses to tone pairs with invariant frequency (STD) show power changes in the delta-theta range. Middle row: ii. Responses to tone pairs with a frequency change in the second tone (DEV) show enhanced delta-theta power at the second tone, relative to STD responses, particularly in the Right auditory region in the Control condition. Third row: Difference plots between STD and DEV responses show a right lateralized increase in power in the Control Rate (A.iii) and bilateral power difference in the Rapid Rate (B.iii). Significant differences between STD and DEV response in the time-frequency domain are shown in black outline. (Adapted from [43]).

DISCUSSION:
The research method described here describes how to facilitate a deeper understanding of spectrotemporal dynamics and anatomical location of high-density auditory-evoked EEG and ERP brain responses in infants. There are four critical steps within this protocol that facilitate analysis. First, proper net application and positioning with minimal caregiver and infant distress is the foundation for recording clean EEG in non-sedated paradigms. Proper head measurement and net size selection as well as the use of a net assistant and entertainer during the application process is key to accomplishing this step. Second, it is important to establish a calm, quiet and playful atmosphere for the family during the testing session, a condition facilitated by the primary tester, net assistant and the entertainer, who engages the infant in quiet play. Third, for data analysis, it is critical that age-appropriate MRI head models be used for source localization. The head size, bone and skin and cerebrospinal space must be accurate for the age tested in order to obtain the most precise localization results. Finally, for cortical responses in general, it is also critical that a high-density net be used (e.g. at least 64 channels of data) in order to optimize the chances of obtaining low-artifact recordings.

One limitation of this technique is that source localization of EEG data is not the gold standard for site of activity tests. One must keep in mind that the forward model of localization even with the best head models and measurements are still *estimates* of activity location. Therefore, it is essential to design the experiment in such a way that information regarding source activity may be compared across experimental conditions or groups. In addition, infant testing in general and in particular, longitudinal study may be fraught with incomplete or missing data sets. Solutions to this problem are to a) maintain relationships with participating families; b) optimize a quiet, calm recording atmosphere for the infant and caregiver; and c) overestimate the subject pool. In our hands, with an experienced pediatric team, we have attained low dropout and minimal data loss rates. In a longitudinal sample of 211 infant recording sessions with 57 participants we show 98.6% data retention (e.g. 208 sessions that resulted in usable data) and a 10% drop out rate (e.g. 6 participants were unable to continue after beginning the experiment). An advantage of EEG over other techniques, such as MEG and NIRS, is that subcortically biased activity is accessible with different filter bands. In addition, it is easier to control for movement as the electrodes travel with the head.

529
530   Once this protocol is mastered, the experimental applications of infant EEG and oscillatory
531   dynamics are abundant. It is clear that we must first understand typically developing cortical
532   networks in order to identify those that are atypically organized. This suggests the need for the
533   creation of a model in which the integrity of early auditory processing mechanisms (including
534   oscillations) plays a role in the generation and plasticity of sound representation as auditory
535   experiences are incorporated and, ideally, learned. According to this model, nonlinguistic
536   processing deficits may be associated with symptoms years, or in some cases decades, before
537   formal diagnosis occurs.
538
539   Future investigations are needed to understand further details, including the function of
540   frequency-band-specific oscillatory dynamics, cross-frequency phase coupling and regional
541   inhibitory/excitatory patterns across early development. In addition, subcortical activity and
542   testing in different states, such as sleep, are needed to give a more complete picture of typical
543   development. We believe research with this technique will provide important insight into the
544   process by which "neurotypical" and atypical oscillatory dynamics organize and interact with
545   emerging cognitive and language abilities.
546

557

558   **REFERENCES:**
559   1.   Gou, Z., Choudhury, N., Benasich, A.A. Resting frontal gamma power at 16, 24 and 36
560   months predicts individual differences in language and cognition at 4 and 5 years. *Behavioural
561   brain research.* **220,** 263-270, doi: 10.1016/j.bbr.2011.01.048 (2011).
562   2.   Uhlhaas, P.J., Roux, F., Rodriguez, E., Rotarska-Jagiela, A., Singer, W. Neural synchrony
563   and the development of cortical networks. *Trends in cognitive sciences.* **14,** 72-80, doi:
564   10.1016/j.tics.2009.12.002 (2010).
565   3.   Singer, W. Development and plasticity of cortical processing architectures. *Science.* **270,**
566   758-764 (1995).
567   4.   Singer, W. Mechanisms of experience dependent self-organization of neuronal
568   assemblies in the mammalian visual system. *Archivos de biologia y medicina experimentales.*
569   **16,** 317-327 (1983).
570   5.   Lakatos, P. *et al.* An oscillatory hierarchy controlling neuronal excitability and stimulus
571   processing in the auditory cortex. *Journal of neurophysiology.* **94,** 1904-1911, doi:
572   10.1152/jn.00263.2005 (2005).

573   6.   Jacobs, J., Kahana, M.J., Ekstrom, A.D., Fried, I. Brain oscillations control timing of single-
574   neuron activity in humans. *The Journal of neuroscience.* **27,** 3839-3844, doi:
575   10.1523/JNEUROSCI.4636-06.2007 (2007).
576   7.   Schroeder, C.E. & Lakatos, P. Low-frequency neuronal oscillations as instruments of
577   sensory selection. *Trends Neurosci.* **32,** 9-18, doi: 10.1016/j.tins.2008.09.012 (2009).
578   8.   Schroeder, C.E., Lakatos, P., Kajikawa, Y., Partan, S., Puce, A. Neuronal oscillations and
579   visual amplification of speech. *Trends in cognitive sciences.* **12,** 106-113,
580   doi:10.1016/j.tics.2008.01.002 (2008).
581   9.   Basar, E. Oscillations in "brain-body-mind"-A holistic view including the autonomous
582   system. *Brain Res.* **1235,** 2-11, doi: 10.1016/j.brainres.2008.06.102 (2008).
583   10.   Buzsaki, G. Large-scale recording of neuronal ensembles. *Nature neuroscience.* **7,** 446-
584   451, doi: 10.1038/nn1233 (2004).
585   11.   Aslin, R.N. Discrimination of frequency transitions by human infants. *The Journal of the
586   Acoustical Society of America.* **86,** 582-590 (1989).
587   12.   Eilers, R.E., Morse, P.A., Gavin, W.J., Oller, D.K. Discrimination of voice onset time in
588   infancy. *The Journal of the Acoustical Society of America.* **70,** 955-965 (1981).
589   13.   Irwin, R.J., Ball, A.K., Kay, N., Stillman, J.A., Rosser, J. The development of auditory
590   temporal acuity in children. *Child development.* **56,** 614-620 (1985).
591   14.   Jusczyk, P.W., Pisoni, D.B., Walley, A., Murray, J. Discimination of relative onset time of
592   two-component tones by infants. *The Journal of the Acoustical Society of America.* **67,** 262-270
593   (1980).
594   15.   Bishop, D.V.M., Hardiman, M.J., Barry, J.G. Auditory Deficit as a Consequence Rather
595   than Endophenotype of Specific Language Impairment: Electrophysiological Evidence. *Plos One.*
596   **7,** e35851, doi: 10.1371/journal.pone.0035851 (2012).
597   16.   Benasich, A.A. *et al.* The infant as a prelinguistic model for language learning
598   impairments: Predicting from event-related potentials to behavior. *Neuropsychologia.* **44,** 396-
599   411, doi: 10.1016/j.neuropsychologia.2005.06.004 (2006).
600   17.   Choudhury, N. & Benasich, A.A. Maturation of auditory evoked potentials from 6 to 48
601   months: prediction to 3 and 4 year language and cognitive abilities. *Clinical neurophysiology :
602   official journal of the International Federation of Clinical Neurophysiology.* **122,** 320-338, doi:
603   10.1016/j.clinph.2010.05.035 (2011).
604   18.   Lakatos, P. *et al.* Timing of pure tone and noise-evoked responses in macaque auditory
605   cortex. *Neuroreport.* **16,** 933-937 (2005).
606   19.   Shah, A.S. *et al.* Neural dynamics and the fundamental mechanisms of event-related
607   brain potentials. *Cerebral cortex.* **14,** 476-483, doi:10.1093/cercor/bhh009 (2004).
608   20.   Fries, P. A mechanism for cognitive dynamics: neuronal communication through
609   neuronal coherence. *Trends Cogn Sci.* **9,** 474-480, doi: 10.1016/j.tics.2005.08.011 (2005).
610   21.   Buzsaki, G. *Rhythms of the Brain.* Oxford University Press, New York (2006).
611   22.   Buzsaki, G. & Draguhn, A. Neuronal oscillations in cortical networks. *Science.* **304,** 1926-
612   1929, doi: 10.1126/science.1099745 (2004).
613   23.   Whittingstall, K. & Logothetis, N.K. Frequency-band coupling in surface EEG reflects
614   spiking activity in monkey visual cortex. *Neuron.* **64,** 281-289, doi:
615   10.1016/j.neuron.2009.08.016 (2009).
616   24.   Ortiz-Mantilla, S., Hamalainen, J.A., Musacchia, G., Benasich, A.A. Enhancement of

617 gamma oscillations indicates preferential processing of native over foreign phonemic contrasts
618 in infants. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* **33,**
619 18746-18754, doi: 10.1523/JNEUROSCI.3260-13.2013 (2013).

620 25. Palmer, S.B., Fais, L., Golinkoff, R.M., Werker, J.F. Perceptual narrowing of linguistic sign
621 occurs in the 1st year of life. *Child development.* **83,** 543-553, doi: 10.1111/j.1467-
622 8624.2011.01715.x (2012).

623 26. Werker, J.F. & Tees, R.C. Speech perception as a window for understanding plasticity
624 and commitment in language systems of the brain. *Dev Psychobiol.* **46,** 233-251, doi:
625 10.1002/Dev.20060 (2005).

626 27. Benasich, A.A. Impaired processing of brief, rapidly presented auditory cues in infants
627 with a family history of autoimmune disorder. *Developmental neuropsychology.* **22,** 351-372,
628 doi: 10.1207/S15326942dn2201_2 (2002).

629 28. Benasich, A.A. & Tallal, P. Infant discrimination of rapid auditory cues predicts later
630 language impairment. *Behavioural brain research.* **136,** 31-49 (2002).

631 29. Lopez-Calderon, J. & Luck, S.J. ERPLAB: an open-source toolbox for the analysis of event-
632 related potentials. *Front Hum.Neurosci.* **8,** 213, doi: 10.3389/fnhum.2014.00213[(2014).

633 30. Delorme, A., Sejnowski, T., Makeig, S. Enhanced detection of artifacts in EEG data using
634 higher-order statistics and independent component analysis. *NeuroImage.* **34** (4), 1443-1449,
635 doi: 10.1016/j.neuroimage.2006.11.004 (2007).

636 31. Hamalainen, J.A., Ortiz-Mantilla, S., Benasich, A.A. Source localization of event-related
637 potentials to pitch change mapped onto age-appropriate MRIs at 6 months of age. *NeuroImage.*
638 **54,** 1910-1918, doi: 10.1016/j.neuroimage.2010.10.016 (2011).

639 32. Ortiz-Mantilla, S., Hamalainen, J.A., Benasich, A.A. Time course of ERP generators to
640 syllables in infants: A source localization study using age-appropriate brain templates.
641 *NeuroImage.* **59,** 3275-3287, doi: 10.1016/j.neuroimage.2011.11.048 (2012).

642 33. Canolty, R.T. *et al.* High gamma power is phase-locked to theta oscillations in human
643 neocortex. *Science.* **313,** 1626-1628, doi: 10.1126/science.1128115 (2006).

644 34. Delorme, A. & Makeig, S. EEGLAB: an open source toolbox for analysis of single-trial EEG
645 dynamics including independent component analysis. *Journal of neuroscience methods.* **134,** 9-
646 21, doi: 10.1016/j.jneumeth.2003.10.009 (2004).

647 35. Delorme, A. *et al.* EEGLAB, SIFT, NFT, BCILAB, and ERICA: new tools for advanced EEG
648 processing. *Computational intelligence and neuroscience.* **2011,** 130714, doi:
649 10.1155/2011/130714 (2011).

650 36. Makeig, S. Auditory event-related dynamics of the EEG spectrum and effects of
651 exposure to tones. *Electroencephalography and clinical neurophysiology.* **86,** 283-293 (1993).

652 37. Hoechstetter, K. *et al.* BESA source coherence: a new method to study cortical
653 oscillatory coupling. *Brain topography.* **16,** 233-238 (2004).

654 38. Papp, N. & Ktonas, P. Critical evaluation of complex demodulation techniques for the
655 quantification of bioelectrical activity. *Biomedical sciences instrumentation.* **13,** 135-145 (1977).

656 39. Yoon, H., Yeom, W., Kang, S., Hong, J., Park, K. A multiple phase demodulation method
657 for high resolution of the laser scanner. *The Review of scientific instruments.* **80,** 056106, doi:
658 10.1063/1.3121218 (2009).

659 40. Wang, Z., Maier, A., Leopold, D.A., Logothetis, N.K., Liang, H. Single-trial evoked
660 potential estimation using wavelets. *Comput Biol Med.* **37** (4), 463-473, doi:

661   10.1016/j.compbiomed.2006.08.011 (2007).

662   41.   Kramer, M.A., Tort, A.B., Kopell, N.J. Sharp edge artifacts and spurious coupling in EEG
663   frequency comodulation measures. *J Neurosci Methods.* **170** (2), 352-357, doi:
664   10.1016/j.jneumeth.2008.01.020 (2008).

665   42.   Kriegeskorte, N., Simmons, W.K., Bellgowan, P.S., Baker, C.I. Circular analysis in systems
666   neuroscience: the dangers of double dipping. *Nat Neurosci.* **12** (5), 535-540, doi:
667   10.1038/nn.2303 (2009).

668   43.   Musacchia, G. *et al.* Oscillatory support for rapid frequency change processing in infants.
669   *Neuropsychologia.* **51** 2812-2824, doi:1 0.1016/j.neuropsychologia.2013.09.006 (2013).

670   44.   deBoer, T., Scott, L.S., & Nelson, C.A. In: *Infant EEG and Event-Related Potentials.*
671   Studies in Developmental Psychology. de Hann, M. ed., Psychology Press, Washington D.C., 5-
672   39 (2007).

673   45.   Basar, E., Schurmann, M., Demiralp, T., Basar-Eroglu, C., Ademoglu, A. Event-related
674   oscillations are 'real brain responses' - wavelet analysis and new strategies. *Int J Psychophysiol.*
675   **39** 91-127, doi: 10.1016/S0167-8760(00)00135-5 (2001).

676
677

Figure1

Step 1. Average Tone-evoked Event-related Potentials
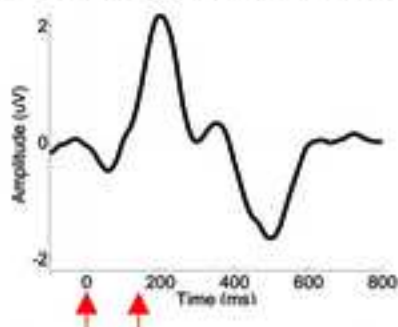
Step 2. Fit peaks to a 2-dipole model
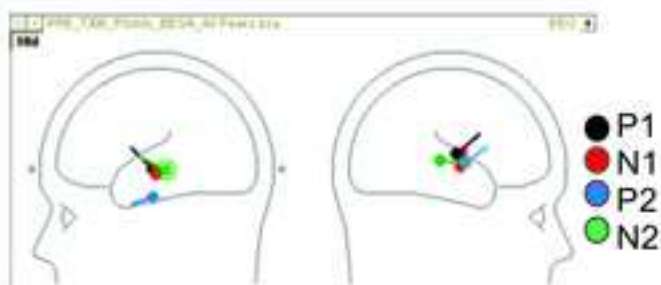
Step 3. Create average source waveforms and solutions
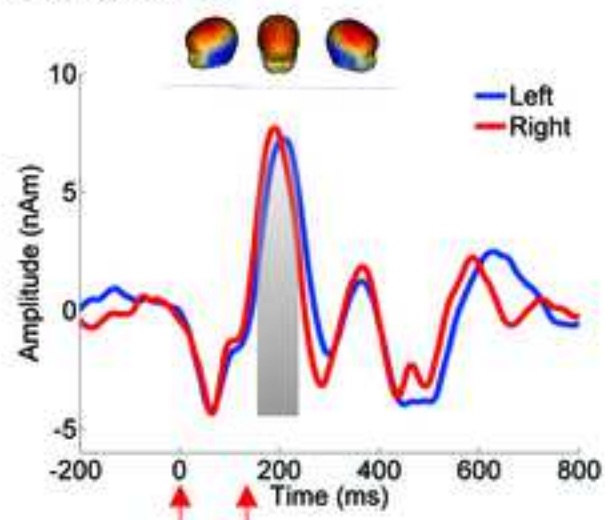
Step 4. Apply source Solution to corresponding Raw EEG
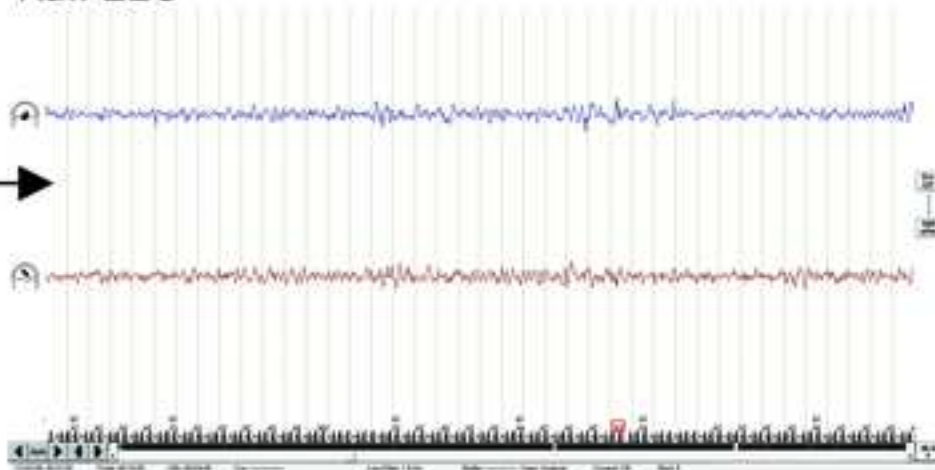
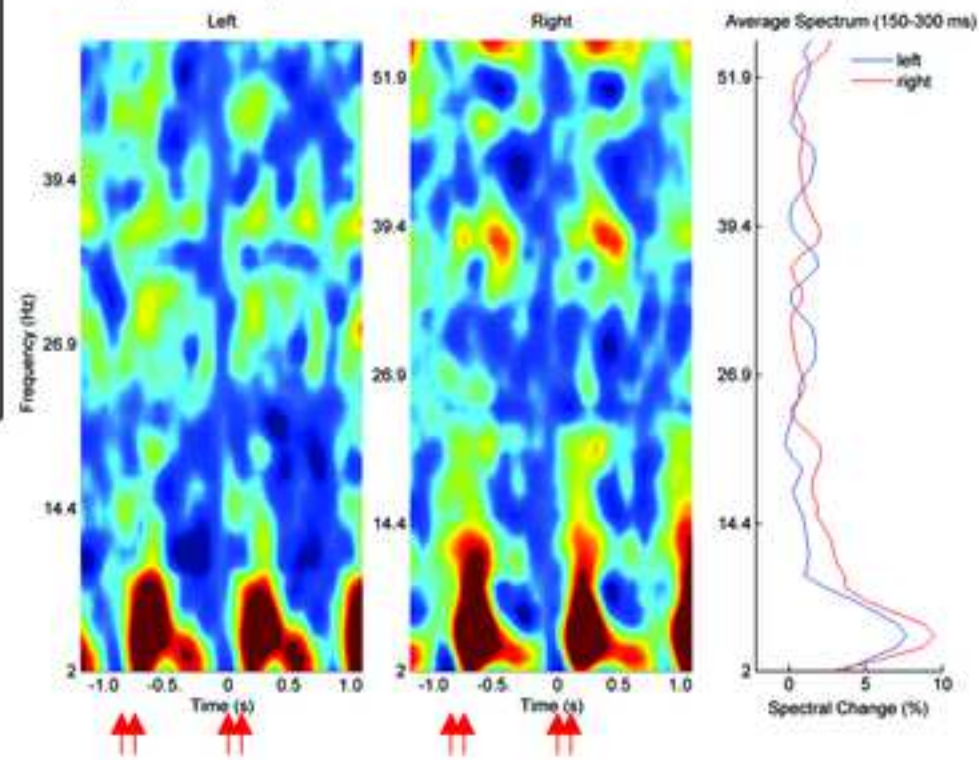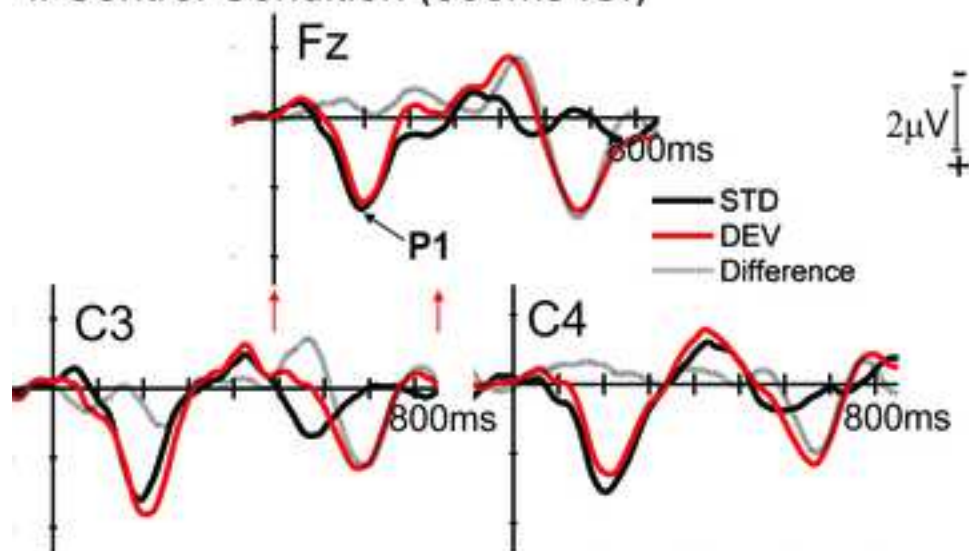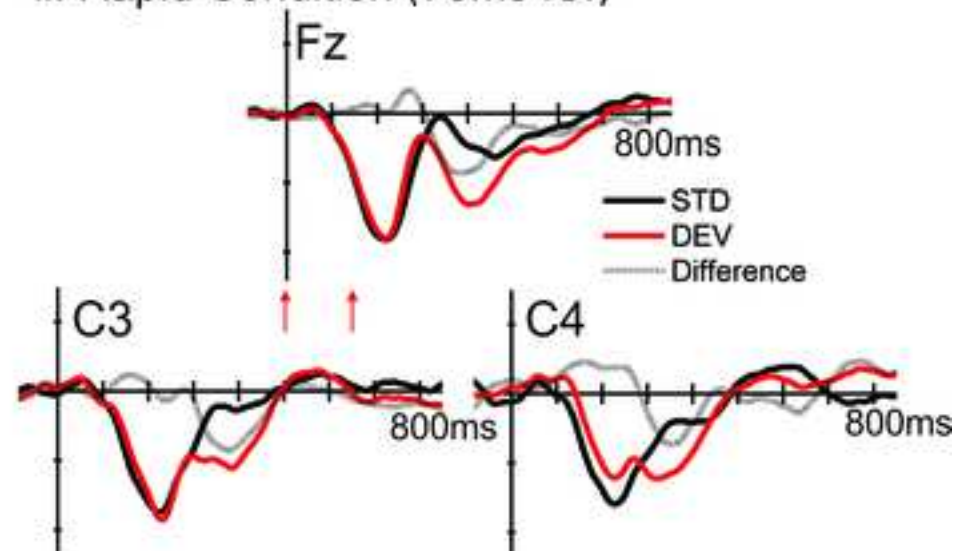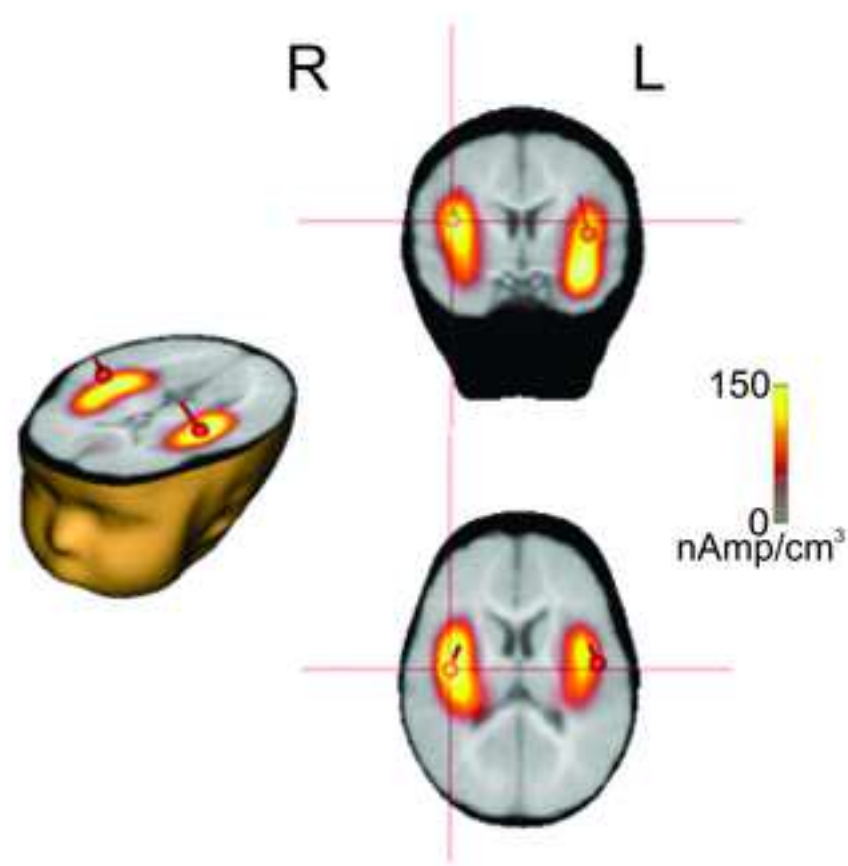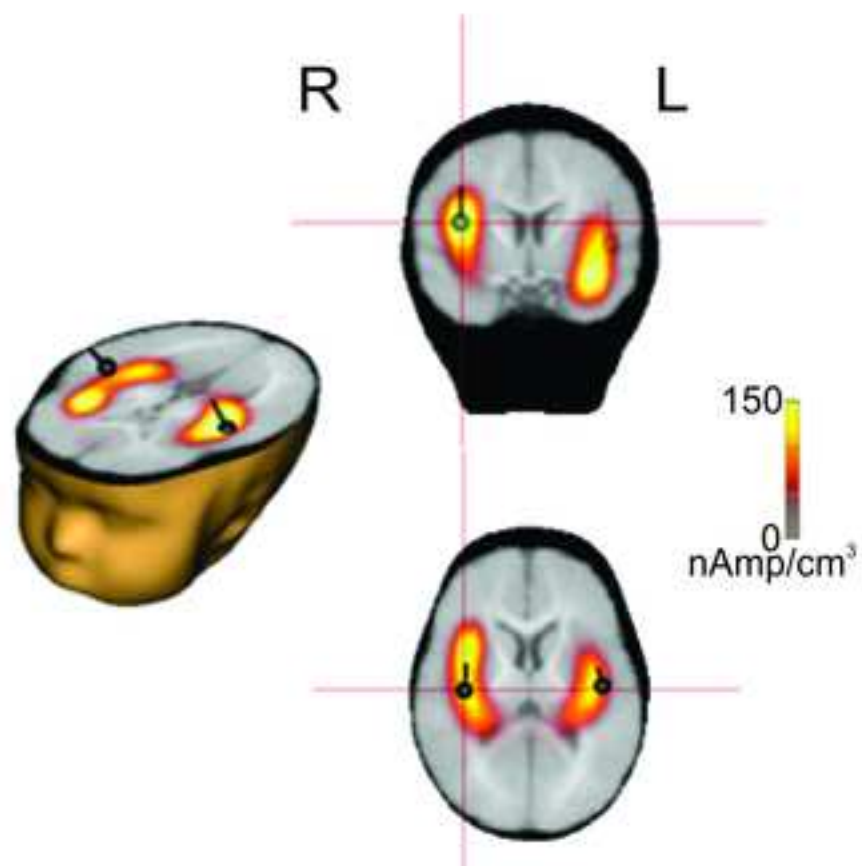Step 5. Compute Event-related Oscillation and Spectrum
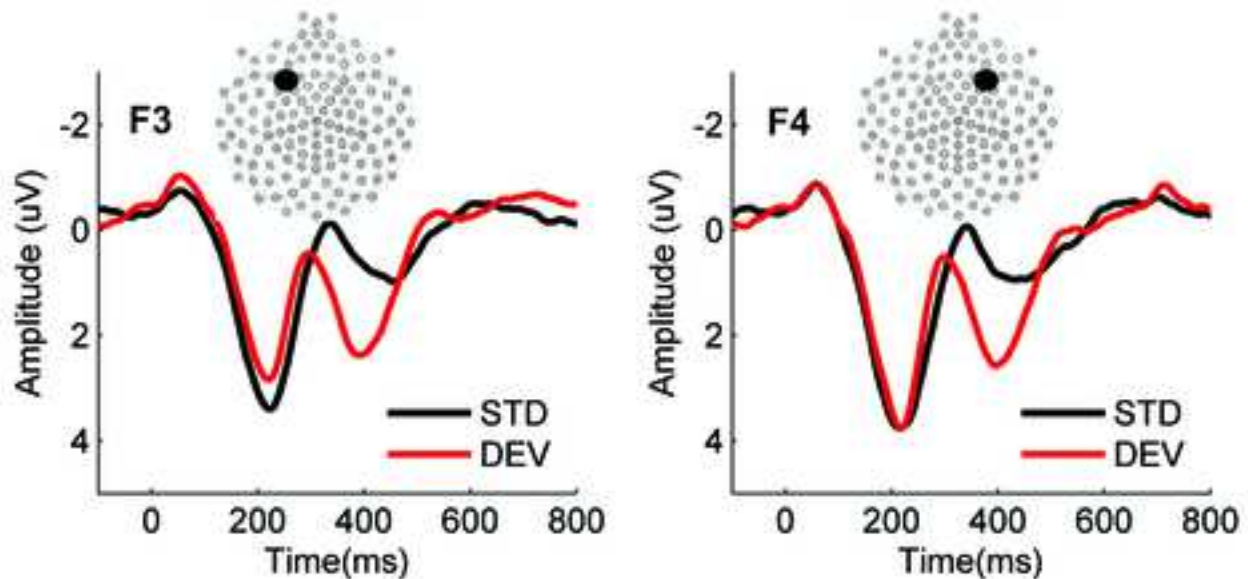
Grand average Event-Related Potentials (n=23)

Grand Average Source Localization (n=23)

i. Control Condition (300ms ISI)
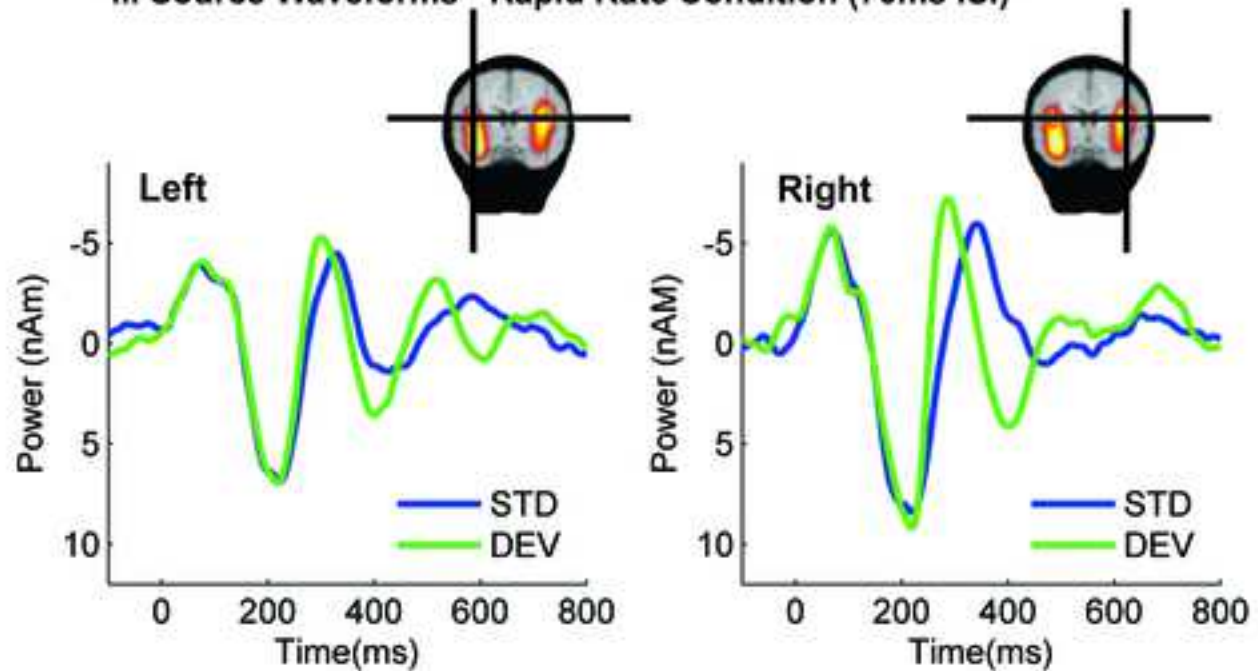
ii. Rapid Condition (70ms ISI)

Grand Average Event-related Potentials and Source Waveforms
(n=23, 4 months old)
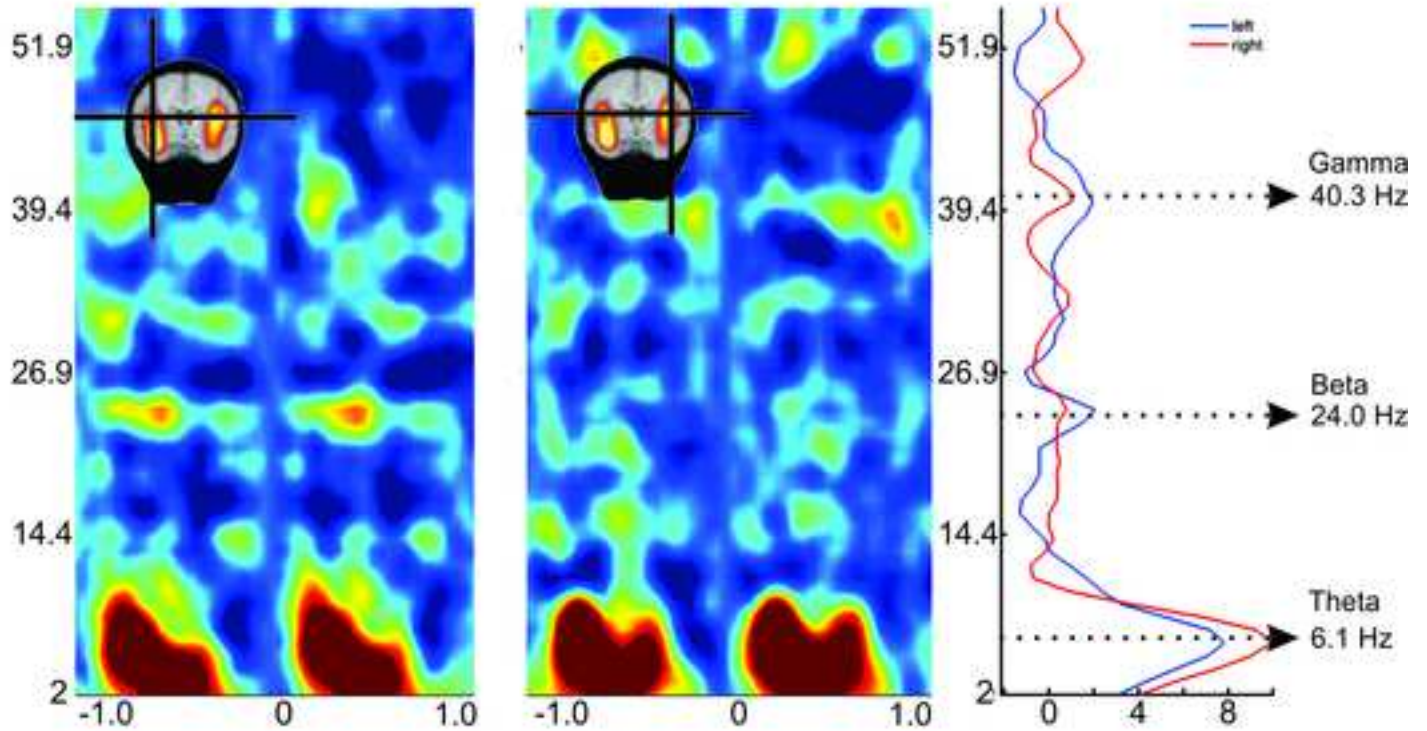
i. ERP - Rapid Rate Condition (70ms ISI)

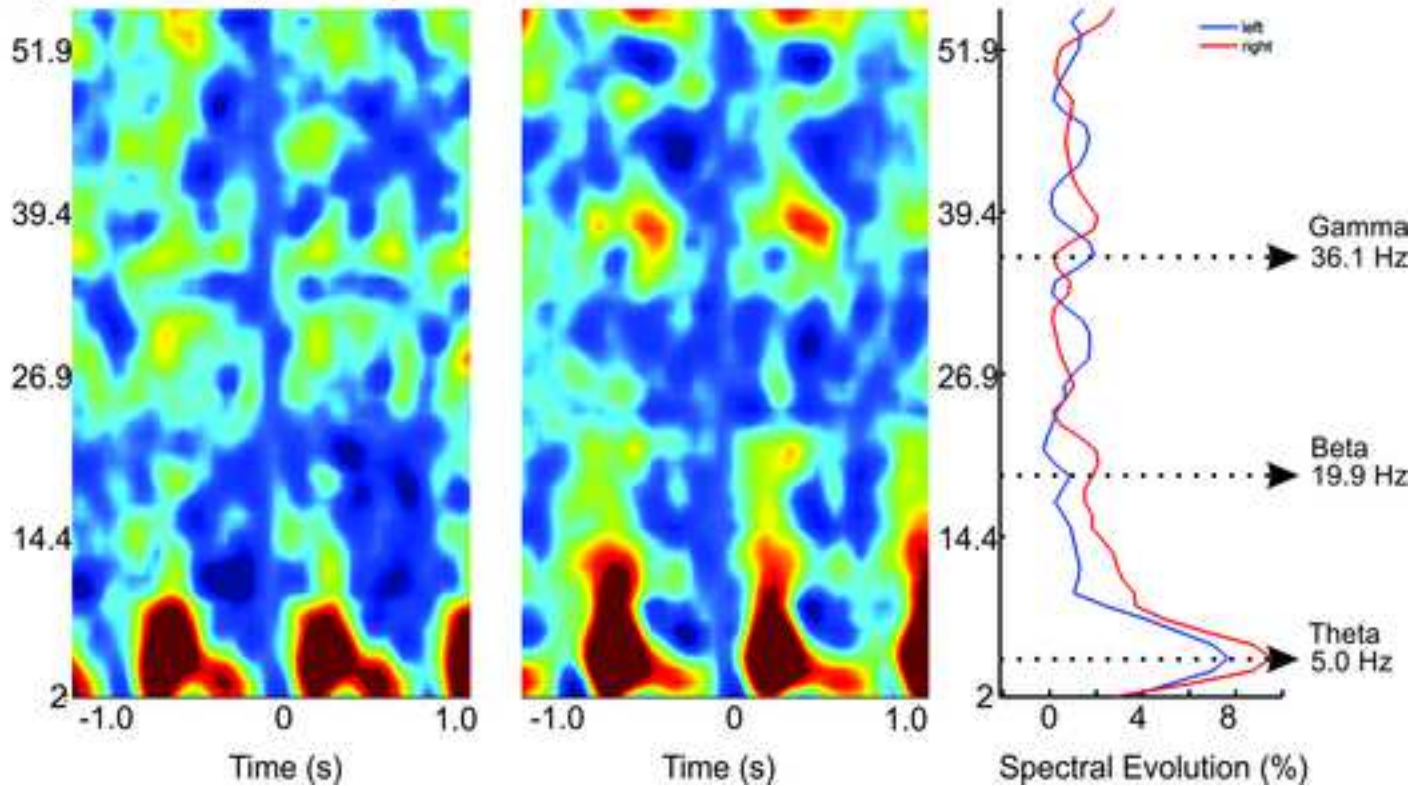ii. Source Waveforms - Rapid Rate Condition (70ms ISI)

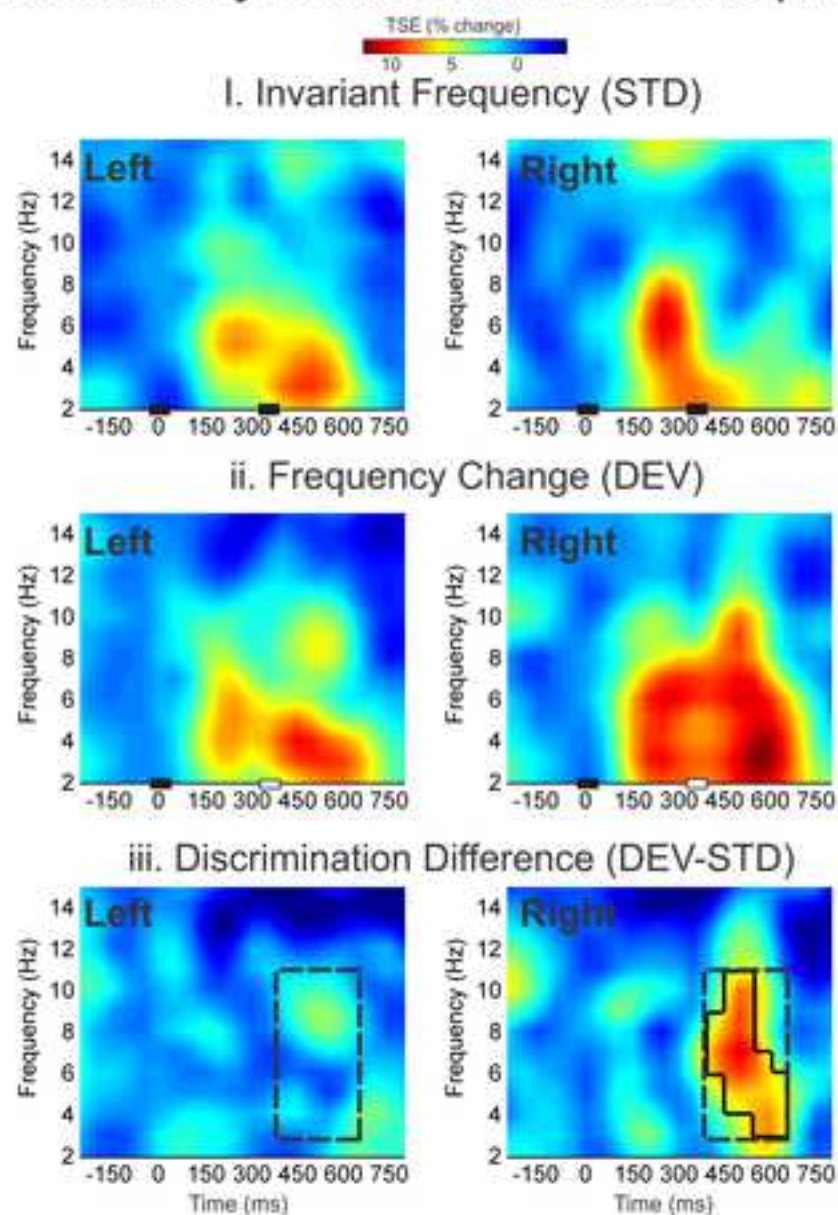**Grand Average Event-related Oscillations from Left and Right Auditory Sources (4 mo. infants)**

A. Oscillatory Power to Control Rate Tone-pairs
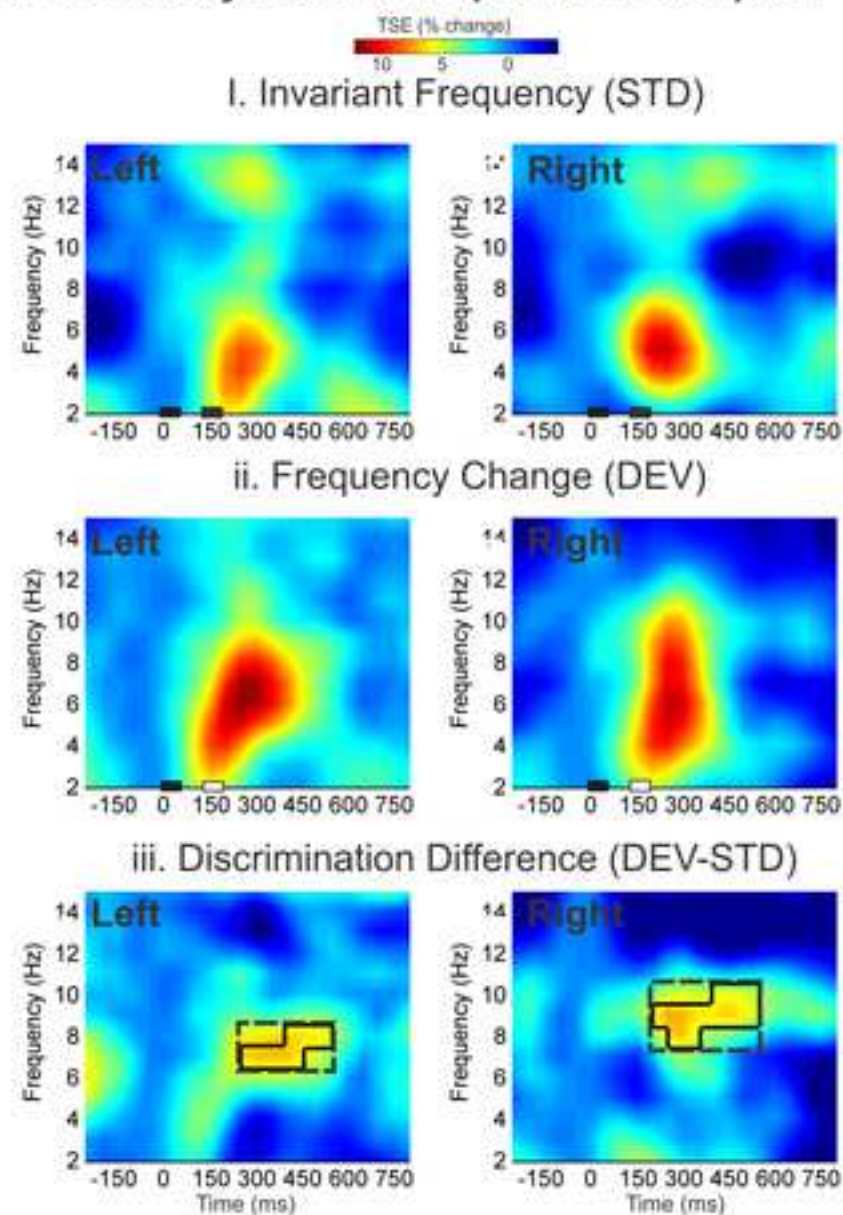B. Oscillatory Power to Rapid Rate Tone-pairs

Excel Spreadsheet- Table of Materials/Equipment

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
EEG Amplifiers	EGI	1301281	
Sensor Nets	EGI	C-GSN-128-1011-110	Sizes of nets vary with age, by month
EEG Recording Software	Net Station	4604200	
Presentation Computer	Dell	4608161	
Presentation Software	Eprime	13102456-50	
Baby bottle warmer	Avent		Target or any baby store
Electrolyte solutuion (Potassium Chl	EGI	A-A-CC-KLL-1000-000	
Coban self-adherent wrap tape	Coban	595573	
Measuring tape			Target or any baby store
Washable Markers			Target or any baby store
Pipettes			Comes with EGI amplifier setup
Analysis Computer	Dell		
Analysis Software I	BESA	3955054	v5.3
Analysis Software II	Brain Voyager	3955054	
	EEGLAB/ERPLAB/		
	MassUnivariate		
Analysis Software III	Toolbox		Freeware MatLAB v2007b
Analysis Software IV	BESA Statistics	3956341	

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Dear Dr. Nguyen,

Thank you for opportunity to revise our manuscript according to editorial comments. We believe we have addressed the final comments thoroughly. We have reviewed our spelling and grammar, omitted methodological descriptions from out figure legends and changed our references to be in citation order.

We look forward to a positive review of this manuscript and moving forward with publication. Please let us know if we can be of further assistance in any way.

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Figure 6, especially.

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