## Participant

The dataset consists of 3 gene-therapy-treated participants (F = 2; age range = 5–14 years; mean = 8.7 years; standard deviation = 4.7 years), and 4 adult control participants (F = ?; age range = ?-? years; mean = ? years; standard deviation = ? years).

## Stimuli

The stimuli consisted of two 250 ms synthetic Mandarin lexical tones, minimally distinguished by their fundamental frequency (F0) contour (tone 2 and tone 4). The synthesis was derived from natural male production data in Reetzke et al.’s 2018 study. The two tones were superimposed over the same syllable /yi/, and only differed in their F0 contour: yi2 low-rising [tone 2], with F0 rising from 109 to 133 Hz; and yi4 high-falling [tone 4], with falling F0 from 140 to 92 Hz. All tones were normalized to the same RMS amplitude at 70 dB SPL and duration at 250 ms.

All stimuli were generated digitally in MATLAB (R2020a; Natick, Massachusetts, USA). The digital stimuli were then presented acoustically in alternating polarities through Bio-logic Navigator Pro System (Natus Medical Incorporated, San Carlos, California). Acoustical stimuli were presented unilaterally to the gene therapy-treated side with ER-3 insert earphones (Etymotic Research, Elk Grove Village, IL).

1. Data collection

In a double-walled sound-attenuating chamber, participants sat in a comfortable chair and were instructed to relax without falling asleep. Frequency following responses (FFR’s) were collected using Ag/AgCl electrodes, with the active electrode placed at the central zero (Cz) point, the reference at the right mastoid, and the ground at the left mastoid. Contact impedance was < 20 kOhm for all electrodes for all recording sessions, and responses were recorded at a sampling rate of 3.2 kHz using Bio-logic Navigator Pro System (Natus Medical Incorporated, San Carlos, California). FFRs were filtered from 80 to 1000 Hz and averaged over a 319.8 ms epoch that began 40 ms prior to stimulus onset. An artifact rejection criterion of ± 35 mV was applied. Multiple block-averages of 500 artifact-free trials were collected in response to the stimulus; thus, after combining blocks, there was an average of 1000 to 5543 artifact-free sweeps of FFR response.

## Data analysis

* 1. Root-mean-square (RMS) and Signal-to-noise ratio (SNR)

The magnitude of the FFR can provide information on the robustness of auditory processing. To determine broadband response magnitude, we calculated the root-mean-squared (RMS) amplitude of the response over a specified time region. For each individual subject’s FFR, 40 ms baseline interval before stimulus onset and 10-260 ms response interval after stimulus onset are calculated. A 10-ms lag addresses general neural transmission delay.

To generate a measure of relative magnitude, we calculate the signal-to-noise ratio (SNR) of the response by dividing the RMS amplitude of the response by the RMS amplitude of the baseline:

* 1. Periodicity (CCAC)

To compare the periodicity fidelity between FFR response and the stimulus, the autocorrelation score of FFR is multiplied with that of the stimulus and normalized with the stimulus autocorrelation score squared. This measure does not require specification of stimulus’ fundamental frequency(F0), thus giving a generalized measure of periodicity.

* 1. Pitch tracking accuracy (F0 tracking) derived from Reetzke et al., 2018

We evaluated the extent to which the FFRs follow F0 changes in the Mandarin lexical tone stimuli by extracting the F0 contour from the averaged FFRs using a periodicity detection short-term autocorrelation algorithm. This algorithm works by sliding a 40-ms window over the time course of the FFR (10 to 260 ms post-stimulus onset). The 40-ms sliding window was shifted in 10 ms steps, to produce a total of 22 overlapping bins. The maximum (peak) autocorrelation value (ranging from 0 to 1) was searched over a lag value of 7 to 11 ms at each bin, a range that encompasses the time-variant periods of the F0 contours for the Mandarin tone stimuli. The peak autocorrelation value, as well as the corresponding lag, were recorded for each bin. The reciprocal of this time lag (or pitch period) was calculated to estimate the F0 for each bin. The resulting frequency values were concatenated to form a 22-point running F0 contour. The short-term autocorrelation algorithm was applied to both the FFRs and the Mandarin tone stimuli. Pitch tracking accuracy metrics were then computed by correlating the F0 contour extracted from the FFRs and the F0 contour extracted from the stimuli.

* 1. Stimulus-to-response correlation (R-S Correlation Maximum and R-S lag)

Morphological similarity of the response with its evoking stimulus is assessed by calculating the stimulus-to-response correlation.

The stimulus was cross correlated with a FFR sliding window (starting at 0-250 ms, temporally shifted at one sample point resolution to later samples). The sliding window time shift (Tau) is limited from 5 to 11 ms.

The maximum correlation coefficient (R-S Correlation Maximum, ranging from 0 to 1) along the sliding process can provide response fidelity to the stimulus. The temporal shift (Tau) for the maximum correlation coefficient is taken as the neural delay (R-S lag). R-S lag can provide an objective measure of broadband timing. Note R-S lag is different from the 10 ms general delay used in calculating RMS and SNR because this measure assesses neural delay in each individual FFR recording.

/Yí/ -- Yi2

A screenshot of a computer generated image

Description automatically generated

Figure: **Yi2** Stimulus vs. Average Control FFR vs. Individual Therapy FFRs

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Initials | Control--B--2000 trials | Control--F--2500 trials | Control--G--2000 trials | Control--H--3000 trials | mean | std | Control--AvgSubj--9500 trials | Therapy--A--3099 trials | Therapy--B--1000 trials | Therapy--C--1500 trials | mean | std | ttest | Cohen's d |
| RMS:Baseline | 0.14 | 0.13 | 0.17 | 0.13 | 0.14 | 0.02 | 0.03 | 0.09 | 0.23 | 0.15 | 0.16 | 0.07 | 0.70 | 0.28 |
| RMS:FFR | 3.98 | 3.01 | 2.05 | 0.80 | 2.46 | 1.36 | 0.84 | 0.32 | 0.61 | 0.39 | 0.44 | 0.15 | 0.05 | 2.08 |
| SNR(dB) | 28.73 | 23.16 | 11.88 | 6.26 | 17.51 | 10.26 | 28.73 | 3.64 | 2.67 | 2.56 | 2.96 | 0.60 | 0.06 | 2.00 |
| CCAC | 1.02 | 1.03 | 1.03 | 0.99 | 1.02 | 0.02 | 1.02 | 1.00 | 0.87 | 0.86 | 0.91 | 0.08 | 0.04 | 1.92 |
| F0 tracking | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.00 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.00 | 0.71 | 0.32 |
| R-S Lag | 7.19 | 7.50 | 7.50 | 7.50 | 7.42 | 0.16 | 7.19 | 10.94 | 6.88 | 6.56 | 8.13 | 2.44 | 0.58 | 0.41 |
| R-S Correlation Max | 0.03 | 0.03 | 0.03 | 0.04 | 0.03 | 0.00 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.00 | 0.05 | 2.11 |

Table: **Yi2** FFR data

/Yì/ --Yi4

A screenshot of a computer screen

Description automatically generated

Figure: **Yi4** Stimulus vs. Average Control FFR vs. Individual Therapy FFRs

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Yi4 | Control--B--2000 trials | Control--F--2534 trials | Control--G--2500 trials | Control--H--2500 trials | mean | std | Control--AvgSubj--9534 trials | Therapy--A--5543 trials | Therapy--B--1113 trials | Therapy--C--1000 trials | mean | std | ttest | Cohen's d |
| RMS:Baseline | 0.11 | 0.11 | 0.11 | 0.14 | 0.12 | 0.02 | 0.02 | 0.04 | 0.17 | 0.18 | 0.13 | 0.08 | 0.83 | 0.16 |
| RMS:FFR | 5.36 | 5.13 | 3.57 | 3.28 | 4.34 | 1.06 | 1.13 | 0.05 | 0.22 | 0.24 | 0.17 | 0.10 | 0.00 | 5.53 |
| SNR(dB) | 47.66 | 46.11 | 33.80 | 22.83 | 37.60 | 11.64 | 47.66 | 1.40 | 1.30 | 1.37 | 1.35 | 0.05 | 0.00 | 4.41 |
| CCAC | 1.08 | 1.06 | 1.08 | 1.07 | 1.07 | 0.01 | 1.08 | 0.61 | 0.35 | 0.33 | 0.43 | 0.15 | 0.00 | 5.88 |
| F0 tracking | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 1.00 | 0.99 | 0.86 | 0.98 | 0.95 | 0.07 | 0.21 | 0.97 |
| R-S Lag | 7.19 | 7.19 | 7.19 | 7.19 | 7.19 | 0.00 | 7.19 | 10.00 | 6.56 | 10.00 | 8.85 | 1.98 | 0.14 | 1.19 |
| R-S Correlation Max | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.00 | 0.14 | 0.10 | 0.06 | 0.06 | 0.07 | 0.02 | 0.00 | 4.97 |

Table: **Yi4** FFR data