Rescuing auditory temporal processing with a novel augmented acoustic environment in a mouse model of congenital SNHL

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Running Head: Improved Temporal Processing Following AAE Exposure

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Abstract

Congenital sensorineural hearing loss (SNHL) affects thousands of infants each year and results in significant delays in speech and language development. Previous studies have shown that early exposure to a simple augmented acoustic environment (AAE) can limit the effects of progressive SNHL on hearing sensitivity. However, SNHL is also accompanied by "hidden hearing loss" that is not assessed on standard audiological exams, such as reduced temporal processing acuity. To assess whether sound therapy may improve these hidden deficits, a mouse model of congenital SNHL was exposed to simple or temporally complex AAE. Peripheral function and sound sensitivity in auditory midbrain neurons improved following exposure to both types of AAE. However, only exposure to a novel, temporally complex AAE significantly improved a measure of temporal processing acuity, neural gap-in-noise detection in the auditory midbrain. These experiments suggest that targeted sound therapy may improve hearing outcomes for children suffering from congenital SNHL.

Keywords: Augmented Acoustic Environment, Temporal Processing, Congenital SNHL, Inferior Colliculus

2 **Introduction**

3   Early childhood sensorineural hearing loss (SNHL) is a common neurosensory disability

4 causing significant medical, social and financial hardship.   The prevalence of moderate-to-

5 profound SNHL in children (> 40 dB) is roughly 3 in 1,000, with up to 10% have hearing loss that

6 is considered "profound" [1-4]. There are numerous causes of congenital or acquired sensorineural

7 hearing loss, including genetic factors, infectious diseases, or environmental toxins.   Beyond

8 hearing threshold deficits seen in children with SNHL, studies have also shown functional deficits

9 in the development of speech and language processing[5-9].   Impairments in speech perception,

10 which may give rise to these functional deficits, have been associated with restricted encoding of

11 auditory temporal cues [10].

12   Psychoacousticians have used the gap detection paradigm to evaluate temporal processing

13 acuity of sounds for more than 30 years. Gap detection acuity may underlie perceptual boundaries

14 in natural language, such as voiced versus voiceless speech sounds. Minimal gap thresholds

15 (MGT) appear to determine the perceptual boundary in the continuum of voice onset times

16 (VOTs), the intervals between consonant release and the start of vocal cord vibration in consonant-

17 vowel transitions [11]. Gap detection acuity is also linked to speech recognition abilities [12], as well

18 as normal language development [13, 14].   In animal models, gap detection can be assessed using

19 several different behavioral techniques [15, 16], and can also be measured neurophysiologically to

20 assess neural sound encoding. Interestingly, nearly all mammals have similar behavioral MGTs,

21 which are on the order of 2-3 ms; the lowest neural MGTs approximate these behaviorally-assessed

22 MGTs [17].

23   There are several mouse models of congenital SNHL that mimic the different types and

24 progression of hearing loss that occur in humans. The DBA strain, the oldest inbred mouse strain

25 [18], contains a mutation in the gene, *Cdh23* [19], as well as a nucleotide substitution in *Fscn2* that is

26 the cause of the *ahl8* quantitative trait locus [20, 21]. This strain shows a rapid, progressive loss of

27 peripheral function beginning at the onset of hearing [22, 23], displaying many of the audiometric

28 characteristics found in infants with progressive SNHL[24]. DBA mice have early and rapid loss of

29 outer hair cell (OHC) function in a base to apex progression, as measured by distortion product

30 otoacoustic emission (DPOAE) thresholds [25].

31   Previous studies have shown that when newborn DBA mice are exposed to broadband

32 sounds daily during 12-hour on/off cycles, improvements are seen in peripheral function [26, 27],

33 preserving hearing sensitivity and limiting hair cell loss [28]. The mechanism through which the

34 slowing of the degenerative processes occurs is unknown but perhaps the AAE maintains afferent

35 neuronal input to the central auditory system (CAS) [29]. An augmented acoustic environment

36 (AAE) exposure also limits neuronal loss in the cochlear nucleus [28] and expands the frequency

37 range to which IC neurons are sensitive across the dorsoventral axis compared to non-exposed

38 mice [30]. When normal-hearing, young adult CBA mice are exposed to AAE no effects, positive

39 or negative, are observed [31]. Clearly, in mouse models of congenital SNHL, AAE exposure shows

40 promise in ameliorating the effects of rapid, progressive SNHL on loss of hearing sensitivity.

41 However, whether AAE exposure can ameliorate other aspects of auditory dysfunction associated

42 with SNHL has yet to be studied. The goal of the current study was to test the hypothesis that

43 exposure to targeted AAE having complex temporal sound features would improve neural

44 correlates of gap encoding in the CAS.

45

46 **Materials and Methods**

47 *Animal Model*

48   The DBA/2J mouse strain has served as a mouse model of early-onset severe hearing loss

49   for over 4 decades [22, 23, 32]. Founder breeding pairs (JAX 000671) were obtained from Jackson

50   Labs (Bar Harbor, ME) and our colony was maintained in micro-isolator facilities in the

51   institutional vivarium. Mice were housed in rodent micro-isolator cages and provided *ad lib* food

52   and water. Lights in the room were on a 12-hr light/dark cycle. Cages were changed at least weekly

53   and mice were monitored for signs of distress by trained vivarium technicians. Breeder pairs were

54   kept separate from experimental mice; only nulliparous mice were used for experiments. Both

55   control and exposed pups were weaned into gender-separated cages between ages postnatal day

56   (P) P21 and P28. All mice in this study were between 1st and 4th generation Jackson Labs breeder

57   mice offspring. All procedures were approved by the University of Rochester's Committee on

58   Animal Resources and in strict accordance with the National Institutes of Health Guide for the

59   Care and Use of Animals.

60

61   *AAE Exposure*

62   Mice were exposed using the same amplifier and sound source as described previously [26]

63   (generously provided by Dr. Jeremy Turner). Cages were housed inside a sound-attenuating

64   chamber (~ 3 feet wide x 2 feet deep x 5 feet high) covered with anechoic foam, and the booth

65   itself was housed in a 2-way vivarium room. Stimulus presentation was calibrated *in situ* to 70 dB

66   SPL using a Quest 1900 Sound Level Meter and an ACO Pacific ¼" free-field microphone (Figure

67   1A). The spectrum of regular AAE (R-AAE) exposure was recorded using an HP/Agilent 35665A

68   Spectrum Analyzer (Hewlett Packard). The analysis revealed a wide-band noise $\pm$ 6 dB from 4 –

69   20 kHz (Figure 1B). Ambient noise levels were between 39 – 45 dB SPL in this frequency range.

70   Our novel temporal AAE (T-AAE) stimulus was generated from a subsection of the wav file

71   containing our original stimulus (Figure 1C), with additional silent gaps inserted within the noise

72   bursts, as follows. Random gap durations of 0, 1, 2, 4, 8 or 16 ms were inserted into the wave

73   vector 100 ms into each 200-ms noise burst, and the remaining noise burst was shortened by the

74   same gap duration to preserve the 40% duty cycle. The resulting wave vector was saved and

75   utilized for temporal AAE stimulus presentation (Figure 1C).

76   AAE exposure began just after birth, before the onset of hearing at around P10 [33] matching

77   previous studies in the DBA model [26, 28, 31]. All mice in this study were tested at 30 days after

78   birth, following at least 18 days of AAE exposure. This time point was chosen because control

79   mice of this strain already show significant hearing loss by this time [22, 23, 26].

80

81   *Peripheral Auditory Assessment*

82   Auditory brainstem responses (ABRs) were recorded using BioSigRP software (version

83   4.4.1, Tucker-Davis Technologies, Alachua, FL) interfacing with Tucker-Davis Technologies

84   (TDT) System III hardware. ABR waveforms were recorded in response to tone bursts of 5 ms

85   duration, shaped by a Blackman window. The frequencies tested were 3, 6, 12, 16, 20, 24, 32 and

86   36 kHz for each animal. Stimuli were presented at a rate of 25 / second, with 150 averages per

87   waveform, with replication. Artifact rejection was enabled with a threshold of 7 μV. Each

88   frequency was presented beginning at a level of 80 dB SPL down to 20 dB below threshold, in 5-

89   dB increments. The recorded waveforms were amplified (x10,000), filtered (0.3 – 10 kHz) and

90   digitized. No mice (control or experimental) responded to test frequencies >24 kHz, and therefore

91   these frequencies were omitted from the ABR analysis.

92   Distortion product otoacoustic emissions (DPOAE) amplitudes were measured using

93   custom MATLAB (Mathworks) software interfacing with TDT System III hardware, calibrated

94 similar to the ABR acquisition hardware. The speaker transduction tube / ER10B microphone

95 apparatus was lowered into the ear canal of the anesthetized mouse using a micromanipulator under

96 microscopic examination. Two separate placements & recordings were completed on each mouse;

97 if the results differed, a third placement and recording was completed. The pair of matching results

98 was averaged during analysis. DPOAE amplitudes were recorded in response to two simultaneous

99 pure-tone bursts (f_1 and f_2) at different frequencies, related with the following ratio: $f_2 / f_1 = 1.25$.

100 The lower-frequency tone (f_1) was presented at 65 dB and the higher-frequency tone (f_2) was

101 presented at 50 dB. The geometric mean presentation frequencies were from $5.6 - 20.5$ kHz.

102 Amplitudes were transformed to the frequency domain and the cubic distortion product ($2f_1 - f_2$)

103 and surrounding noise values were measured. DPOAE responses could not be distinguished from

104 the noise floor above 22 kHz in any group, and these frequencies were not analyzed.

105

106 *Auditory Midbrain Neurophysiology*

107 We recorded neuronal activity in the inferior colliculus (IC) using a 16-channel vertically-

108 oriented electrode (a1x16-3mm-100-177μm^2, NeuroNexus Technologies) with 100 μm spacing

109 between pads and impedances of $1 - 3$ MΩ. Electrodes were positioned over the craniotomy and

110 advanced ventrally by a micromanipulator. The electrical output was amplified, filtered and

111 digitized at 25 kHz in a 1.25 ms time window. Neural activity was automatically determined using

112 a 3:1 SNR.

113 Stimuli were generated using DSP software (OpenEX and RPVDS, TDT) and presented

114 through TDT System III hardware to an electrostatic speaker (TDT ES1). The speaker was located

115 at a 60° azimuth contralateral to the recording site. Stimulus presentation was controlled by

116 custom MATLAB routines interfacing through OpenEx interfaced to an RX6. First, search stimuli

117   were presented to locate responsive units and identify events. These stimuli were band-limited

118   noise bursts (3 – 50 kHz) presented at 70 dB SPL at a rate of 2 / second. Second, tone burst stimuli

119   (25-ms in duration, 10 / sec) were presented to measure frequency response areas (FRA). The

120   range of frequencies used in this study was 2 – 64 kHz (500 Hz increments) and the range of levels

121   was 0 – 85 dB SPL (5 dB increments). Each frequency-level pair was presented five times, with

122   the entire set randomized prior to presentation. Third, to assess gap-in-noise encoding noise bursts

123   of 100 ms (noise burst 1, NB1) and 50 ms (noise burst 2, NB2) were delivered at a rate of 2 / sec.

124   The level was fixed at the start of each run to 80, 70, or 60 dB SPL, as these intensities were

125   predicted to be >20 dB higher than the noise threshold for individual units. Silent gaps in the noise

126   burst were inserted (0.25 msec rise-fall) after the first 100 ms of the first noise burst (NB1), with

127   the gap duration being one of the following: 0, 1, 2, 4, 8, 16, 32, 64, or 96 ms. Continuous

128   background noise (CBN) was used to further test the benefits of AAE exposure. CBN (3-50 kHz)

129   could be applied to a gap series at a fixed level (+6 dB SNR) whereby the silent gap would also be

130   filled with this continuous background noise at a level of 6 dB below the noise carrier. Each gap

131   duration was repeated 50 times, for a total of 500 repetitions (10 gap durations x 50 repetitions per

132   duration).

133

134   *Spike Sorting and Response Measures*

135   Spike waveforms were processed in MATLAB® using the TDT OpenDeveloper ActiveX

136   controls and passed to AutoClass C v3.3.4, an unsupervised Bayesian classification system that

137   seeks a maximum posterior probability classification, developed at the NASA Ames Research

138   Center [34]. AutoClass scans the dataset of voltage–time waveforms according to custom specified

139   spike parameters to produce the best-fit classifications of the data, which may include distinct

140   single- and multi-unit events, as well as noise. To discriminate the signal from noise, the variance

141   of the background noise was estimated as the quartile range of the first five digitization points of

142   the spike waveform, as these are recorded prior to the threshold-crossing event. To avoid

143   overloading AutoClass with excessive noise, which leads to over-classification, this noise measure

144   was used to screen the event waveform data such that only voltage points with absolute values

145   greater than this noise floor were presented for use in the classification. Once the classes had been

146   determined in each channel of data, they were visualized within a custom MATLAB® program

147   and assigned to multi-unit, single-unit, or noise classes. Event classes which were categorized as

148   noise were subsequently discarded, and units with distinct biphasic waveforms and good SNR

149   were classified as single-units. As most channels recorded information elicited from the spiking of

150   two or more neurons, all recordings units in this paper were considered to be multi-unit activity [35].

151   Nonetheless, there was no observation of any consistent differences in the eFRAs between single

152   units and multi-unit clusters.

153   Data analysis was performed as previously described [36]. Frequency response areas (FRAs)

154   were displayed in a custom MATLAB GUI and analyzed with a multi-step procedure using custom

155   software. Frequency receptive fields (FRAs) were then used to determine the best frequency (BF),

156   the frequency with the lowest intensity of driven activity, and tuning sharpness. For units with

157   sound driven activity, assessed within the FRA, neural responses to gap-in-noise were visualized

158   using a custom Matlab GUI, and minimum gap thresholds (MGTs) were determined using

159   previously-published methods [37, 38]. Only gap-responsive units (with MGT $\leq$ 96 ms) for each

160   stimulus condition were included in subsequent analysis of gap detection. Additionally, the

161   analysis focuses on phasic units because tonic units recorded in continuous background noise

162   demonstrated post-excitatory suppression. Due to this post-excitatory suppression, the quiet

163   window responses of tonic units were not strictly a result of the embedded silent gap, making MGT

164   determination highly variable and unreliable.

165

166   *Statistical Analysis*

167   Table 1 reports the number of mice that underwent ABR, DPOAE, and IC recordings, and

168   the number of IC units included in each measure of neural sound processing. Mice with DPOAE

169   recordings were a subset of mice with ABR recordings; however, mice that underwent IC

170   recordings did not always have peripheral assessment. Auditory processing measures are reported

171   as mean ± standard error of the mean and statistical comparisons were made using GraphPad Prism

172   v6.0. The Student's t-test compared differences between two groups, while Analysis of Variance

173   (ANOVA) with Bonferroni *post-hoc* testing compared the effect of one or more variables. The Chi

174   Square or Fischer's Exact test was used to examine differences between observed and expected

175   counts. Significance was set at $p < 0.05$.

176

177   **Results**

178   *Peripheral Auditory Assessment*

179   ABRs were recorded to determine the effect of early AAE exposure beginning at the onset

180   of hearing. ABR thresholds were assessed as a function of Exposure and Frequency (Figure 2A,

181   D). A two-way ANOVA demonstrated significant effects of Exposure ($F = 23.46$, $p < 0.001$),

182   Frequency ($F = 121.10$, $p < 0.001$) and Exposure x Frequency ($F = 5.71$, $p < 0.001$) with post AAE

183   thresholds from exposed mice being significantly improved as compared to control mice. Post-hoc

184   analysis showed significant improvement in ABR thresholds at 12 and 16 kHz following either

185   type of AAE exposure, and no significant differences between the two types of AAE exposure.

186   The magnitude of the difference in ABR thresholds for control and AAE-exposed mice approached

187   30 dB at 16 kHz (Control vs. Regular AAE: 29 dB; Control vs. Temporal AAE: 26 dB), a

188   frequency in the range of the best hearing for CBA mice. While the difference did not reach

189   statistical significance, at 24 kHz we encountered very few mice from the Control group that had

190   observable responses at 80 dB (3 / 20, or 15%), when compared to the Regular AAE (6 / 16, or

191   38%) or Temporal AAE groups (7 / 15, or 46%). Together, these findings indicate that ABR

192   thresholds improved following exposure to both types of AAE (Figure 2A, D), replicating the

193   findings of Turner and Willott (1998). Moreover, the frequency range that showed the most

194   improvement in ABR thresholds was within the region of maximal energy for the AAE exposure

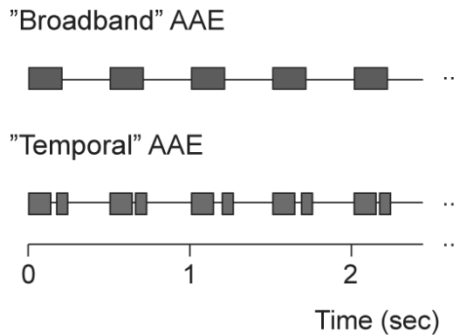195   spectrum and with the frequency region of best hearing sensitivity.

196
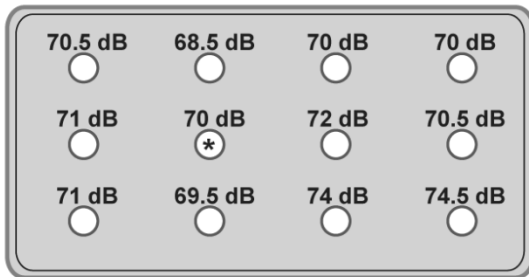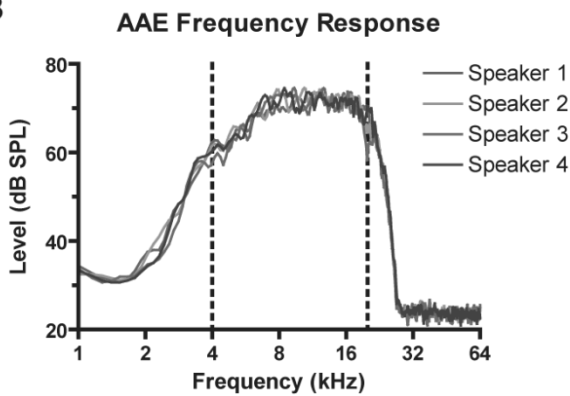
Figure 1. Exposure calibration, stimulus spectrum and temporal pattern. **A**, Sound levels recorded at different points in the cage (circles, spaced approx. 3.5" apart) in response to calibrated AAE stimulus. Asterisk denotes hole calibrated to 70 dB SPL. **B**, The frequency response spectrum of the AAE stimulus, presented through each speaker used during exposure, demonstrates a flat region ($\pm$ 6 dB) from 4 – 20 kHz (indicated with dashed lines). The spectrum was recorded with a ½" ACO Pacific microphone on a Quest 1900 sound level meter output to an HP/Agilent 35665A spectrum analyzer. Each waveform consisted of 30 averages. **C**, Schematic of regular and temporal augmented acoustic environment exposure. Each exposure was presented twice per second, 200 ms per burst, for 12 hrs / day. Temporal AAE stimulus had a silent gap (either 0, 1, 2, 4, 8 or 16 ms in duration) inserted after the first 100 ms.

	Control	Regular AAE	Temporal AAE
Periphery (# Mice)			
ABRs	20	16	15
DPOAEs	13	12	8
Auditory Midbrain			
# Mice	11	11	10
Total # Units Recorded	825	932	904
Frequency Response Areas (%)	574 (69.6)	702 (75.3)	629 (69.6)
80 dB Gap-Responsive (%)	507 (61.5)	595 (63.8)	567 (62.7)
80 dB Phasic Units (%)	242 (29.3)	192 (20.6)	267 (29.5)
80 dB Gap-In-Noise Responsive	173 (21.0)	134 (14.4)	210 (23.2)
70 dB Phasic Units (%)	204 (24.7)	184 (19.7)	234 (25.9)
60 dB Phasic Units (%)	96 (11.6)	170 (18.2)	188 (20.8)

Table 1. Counts of animals tested and units isolated. For peripheral measures, counts are given in terms of the number of animals tested. For central auditory recording, counts are listed in terms of the number of animals as well as the number of units recorded from these animals. Percent values are listed as percent of total units recorded for each exposure type.

207   The amplitude and latency of wave I in the ABR waveform was measured in response to a

208   12 kHz tone presented at 80 dB SPL (Figure 2B, C). Wave I amplitudes (Figure 2B) demonstrated

209   a significant effect of Exposure ($F = 5.94$, $p = 0.0118$). Post-hoc group comparisons reveal wave

210   I amplitudes were significantly greater for temporal AAE-exposed mice than controls (4.3 ± 1.1

211   μV compared to 9.9 ± 0.8 μV, $p < 0.05$). Mean wave I amplitude was also greater for regular

212   AAE-exposed mice (6.7 ± 1.1 μV) than controls, though the difference did not reach significance.

213   Although the mean wave I latencies were physiologically similar across groups, differing by only

214   170 μsec (Control: 2.98 ± 0.03 ms; Regular AAE: 2.96 ± 0.03 ms; Temporal AAE: 3.13 ± 0.03

215   ms; Figure 2C) the one-way ANOVA demonstrated a significant effect of Exposure ($F = 6.81$, p

216     $= 0.007$). Together these findings indicate the temporal AAE exposure had a more substantive

217   effect on wave I ABR measures of cochlear sound processing than regular AAE exposure.

218   DPOAE amplitudes were measured in mice following exposure to regular or temporal

219   AAE versus control environments to assess outer hair cell function (Figure 2E). A two-way

220   ANOVA found significant effects for Exposure ($F = 109.00$, $p < 0.001$), Frequency ($F = 17.98$, p

221   < 0.001) and Exposure x Frequency ($F = 5.25$, $p < 0.001$). Post-hoc group comparisons revealed

222   that DPOAE amplitudes increased at geometric mean frequencies between $10 - 17$ kHz for both

223   groups of AAE exposure relative to control (mean increase for pooled AAE-exposure responses:

224   21.2 ± 1.4 dB, $p < 0.05$). Temporal AAE exposure resulted in an even larger impact on DPOAE

225   amplitudes than regular AAE exposure. Post-hoc group comparisons revealed that DPOAEs

226   elicited by 17.27 and 18.84 kHz tones were significantly larger (by 13 dB) in temporal AAE-

227   exposed animals compared to regular AAE-exposed animals ($p < 0.05$). Thus, similar to the ABR

228   analysis, the DPOAE analysis showed the greatest impact of AAE exposure on cochlear function

229   for stimuli in the frequency region with maximal AAE energy. Additionally, Temporal AAE

230   exposure was moderately more effective at improving DPOAE measures of cochlear function.
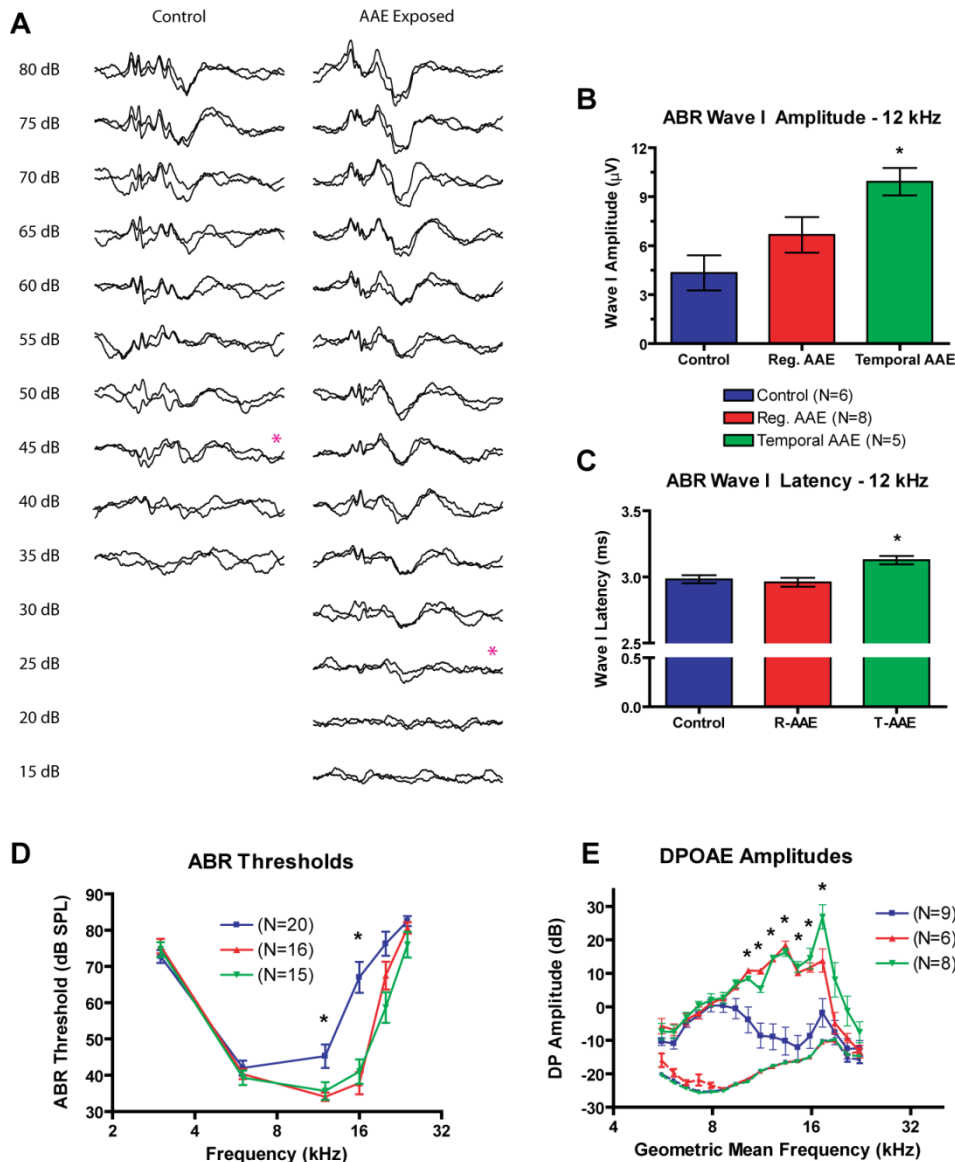
Figure 2. AAE exposure improves peripheral function at P30. **A**, Representative ABR waveforms from a control and AAE-exposed animal at 12 kHz show similar suprathreshold morphology, with an elevated threshold in the control animal. **B**, ABR wave I amplitudes were increased in both exposure types compared to controls (Control *[blue]*: 4.3 ± 1.1 µV; Reg. AAE *[red]*: 6.7 ± 1.1 µV; Temporal AAE *[green]*: 9.9 ± 0.8 µV), though only responses from temporal AAE exposure reached significance ($p < 0.05$). **C**, ABR wave I latencies were similar in magnitude (Control: 2.98 ± 0.03 ms; Reg. AAE: 2.96 ± 0.03 ms; Temporal AAE: 3.13 ± 0.03 ms). Temporal AAE-exposed mice had significantly longer latency compared to control mice ($p < 0.05$) and regular AAE mice ($p < 0.01$). **D**, ABR thresholds were significantly decreased at 12 & 16 kHz following exposure to both types of AAE (Regular = *red*, Temporal = *green*) compared to controls (*blue*, $p < 0.001$). At the frequency of greatest differences (16 kHz), this difference approaches 30 dB. No responses were noted above 24 kHz in any group. **E**, DPOAE amplitudes were increased following exposure to both types of AAE (Regular = *red*, Temporal = *green*) compared to controls (*blue*), with larger increases seen at select frequencies following temporal AAE exposure. Between 10 and 17 kHz,

246   both types of AAE exposure significantly increased amplitudes (mean increase: 21.2 ± 1.4 dB, p
247   < 0.01). Additionally, temporal AAE exposure resulted in a 13 dB amplitude increase over regular
248   AAE exposed mice at two test frequencies (17.27 & 18.84 kHz). Amplitudes could not be
249   distinguished from the noise floor above 22 kHz in any group.

250   *Central Auditory Function*

251   To determine whether AAE exposure can influence neural markers of spectral or temporal

252   auditory processing acuity, we measured the response of IC neurons to sound stimuli in vivo. To

253   our knowledge this is the first description of the effects of AAE on neural coding of complex

254   sounds in the CAS. Exposure to AAE altered the frequency response properties for IC units when

255   compared to units from control, unexposed animals (Figure 3A-D). One-way ANOVAs

256   demonstrated that exposure to AAE resulted in a significant upward shift in BF ($F = 43.16$, $p <$

257   0.001) and a significant improvement in minimum threshold ($F = 15.46$, $p < 0.001$) of neurons

258   from AAE groups. Group comparisons revealed that both types of AAE exposure significantly

259   increased the upward frequency boundary of BFs relative to Control values (Reg. AAE: 14.2 ± 0.2

260   kHz; Temporal AAE: 15.1 ± 0.2 kHz; Control: 12.4 ± 0.2 kHz; $p < 0.001$), with no further

261   differences between regular and temporal AAE exposure groups (Figure 3B). Likewise, both types

262   of AAE exposure significantly improved minimal response threshold, with no further effect of

263   AAE exposure group (Figure 3C). Finally, exposure to either type of AAE sharpened tuning of the

264   FRAs, assessed by measuring Q-values for the FRA between 10 and 40 dB above threshold. One-

265   way ANOVAs demonstrated a significant effect of Exposure for Q_{10} through Q_{40} values (Q_{10}: $F =$

266   28.81; Q_{20}: $F = 43.46$; Q_{30}: $F = 34.09$; Q_{40}: $F = 48.58$; $p < 0.001$). A higher Q-value indicates

267   sharper tuning, and group comparisons showed that Q_{10} through Q_{40} values following either type

268   of AAE exposure were higher than control values (Q_{10} shown in Figure 3D). The mean magnitude

269   of the difference between control and AAE exposure groups at Q_{10} is approximately 1, which at a

270   BF of 12 kHz equates to a bandwidth difference of about 1 kHz (or 25% of the Control group

271   mean). No other significant differences were found with respect to frequency receptive fields

272   between exposure types. These findings indicate that in AAE-exposed mice, IC neurons had lower

273   thresholds and were more responsive to higher frequencies, but were also more narrowly tuned.

274   The type of AAE exposure did not influence these improvements in spectral acuity.
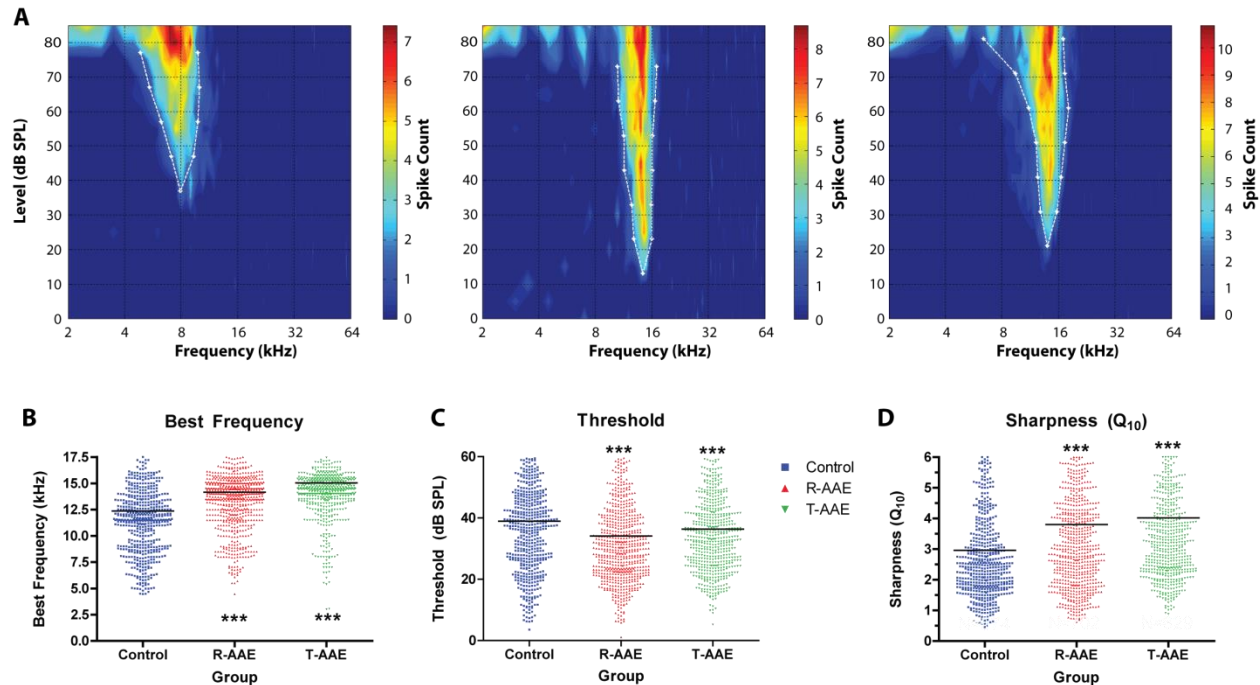
Figure 3. Exposure to AAE changes the frequency responses of IC units. **A**, Example frequency response areas (FRA) are shown from representative control, regular AAE-exposed and temporal AAE-exposed animals. Color-mapped counts indicate the number of spikes per frequency-level pair, with the legend shown on the right. Best frequency (BF) was automatically identified as the frequency with the lowest intensity of drive (minimum threshold, or MT). **B**, Mean best frequencies were significantly increased compared to controls following either type of AAE exposure (Reg. AAE: 14.2 ± 0.2 kHz, Temporal AAE: 15.1 ± 0.2 kHz, Control: 12.4 ± 0.2 kHz; $p < 0.001$). No significant difference was seen between AAE exposure types. **C**, Mean unit thresholds were significantly decreased following either type of AAE exposure (Reg. AAE: 34.2 ± 0.6 dB, Temporal AAE: 35.9 ± 0.5 dB, Control: 38.9 ± 0.7 dB; $p < 0.01$). Again no significant difference was seen between AAE exposure types. **D**, Tuning sharpness was improved with both types of AAE exposure. Q-values computed at 10 dB as well as 20 dB, 30 dB and 40 dB above threshold (data not shown) were significantly increased compared to controls (one-way ANOVA with post-hoc testing, $p < 0.001$ at all levels). No significant differences were seen between the two types of AAE exposure. Graphs in B, C and D were vertically scaled to demonstrate differences in the mean, and thus some data points above the maximum vertical axis value are not shown.

293   Temporal processing acuity was assessed via MGTs, a measure of neural coding of silent

294   gaps embedded in noise. Representative post-stimulus time histograms (PSTHs) of single phasic

295   units in response to different gap durations are shown in Figure 4. Minimum gap thresholds

296   (MGTs) were computed for all phasic units, and units were included in each of the following

297   analyses if the gap threshold for the condition was ≤ 96 ms (Figure 5). For gaps embedded in 80-

298   dB noise carriers, one-way ANOVA demonstrated a significant effect of Exposure ($F = 15.43$, p

299   < 0.001; Figure 5A). Both types of AAE exposure shortened MGTs relative to controls. The mean

300   magnitude of improvement for the regular AAE exposure group was 4.89 ms (33%), and for the

301   temporal AAE exposure group was 6.58 ms (44%). For 70-dB carriers, one-way ANOVA

302   demonstrated a significant effect of Exposure ($F = 12.49$, $p < 0.001$). Again, both types of AAE

303   exposure improved MGTs compared to controls, with greater average improvement seen by mice

304   exposed to temporal AAE (8.10 ms) versus those exposed to regular AAE (5.11 ms). Post-hoc

305   comparison did not show a significant difference between groups exposed to regular versus

306   temporal AAE. For silent gaps embedded in 60-dB SPL carriers, one-way ANOVA demonstrated

307   an effect of Exposure on MGT ($F = 7.31$, $p < 0.001$). Group comparisons showed that mice

308   exposed to temporal AAE had significantly shorter MGTs compared to mice exposed to regular

309   AAE (temporal AAE vs. regular AAE: 15.9 ± 1.2 ms vs. 23.3 ± 1.6 ms, p < 0.01). Control mice

310   also had shorter MGTs compared to mice exposed to regular AAE, though these differences did

311   not reach significance (control vs. regular AAE: 18.6 ± 1.7 ms vs. 23.3 ± 1.6 ms). However, the

312   number of units with detectable minimal gap thresholds was also substantially lower for control

313   mice (96 units) than for either regular (170 units) or temporal (188 units) AAE-exposed mice ($\chi^2(2)$

314    $= 118.08$, $p < 0.001$). In contrast with phasic units, tonic units showed no significant effects of

315   AAE exposure on responses to gap stimuli or MGTs (data not shown). Overall, these findings

316   indicate that gap detection generally improved in phasic units following exposure to both types of

317   AAE, with a trend towards greater improvement seen following exposure to our novel temporal

318   AAE.

319
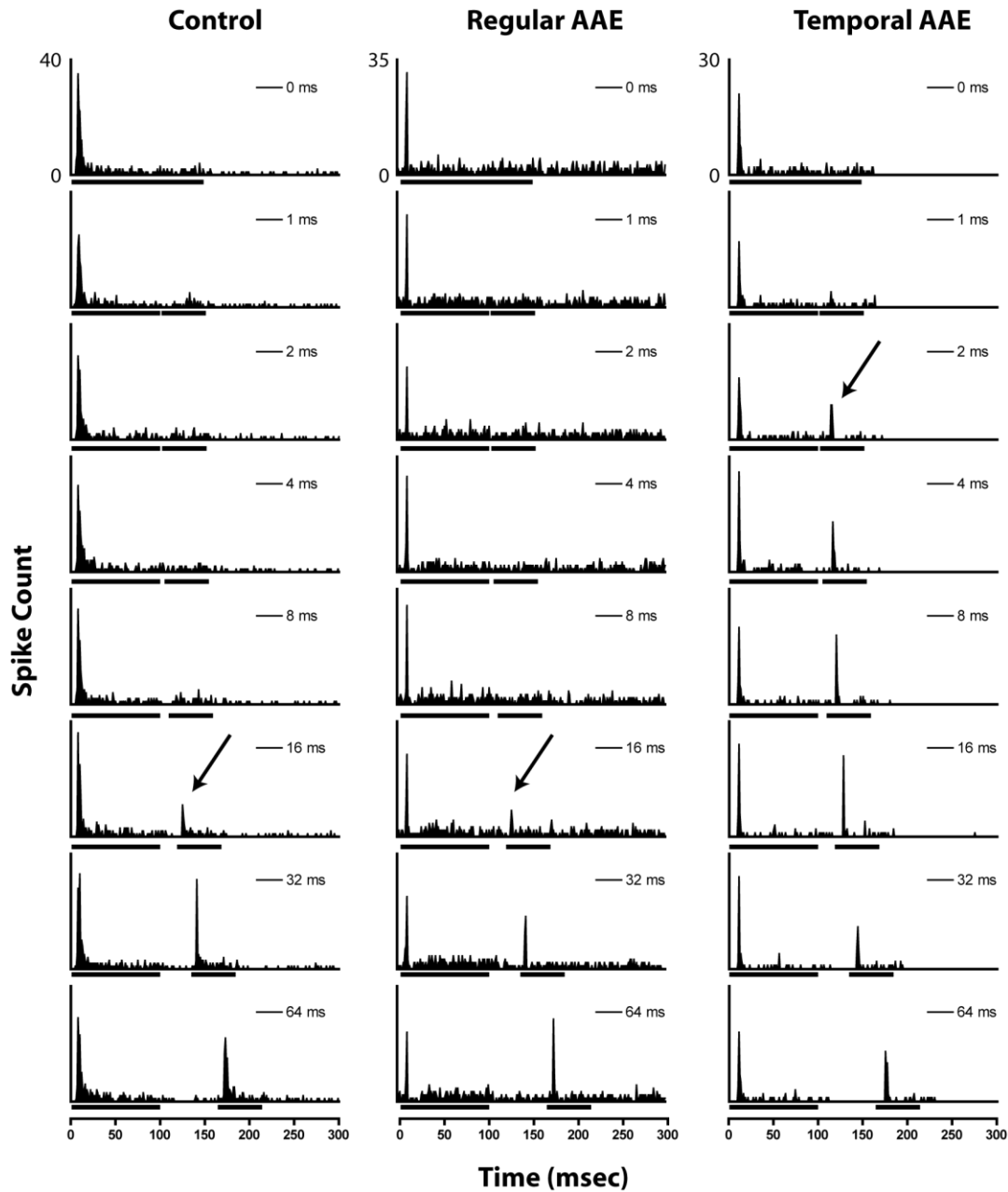
Figure 4. Representative examples of a neural correlate of gap encoding by phasic units from unexposed (*left*), regular AAE-exposed (*center*), and temporal AAE-exposed (*right*) mice. Post stimulus time histograms show spike counts summed over 50 presentation of a gap-in-noise paradigm using a carrier level of 80 dB SPL with gap duration shown in each PSTH. Bars under the x-axis denote noise-burst duration marking the silent gap. Arrows denote the automatically-calculated MGT for each unit.
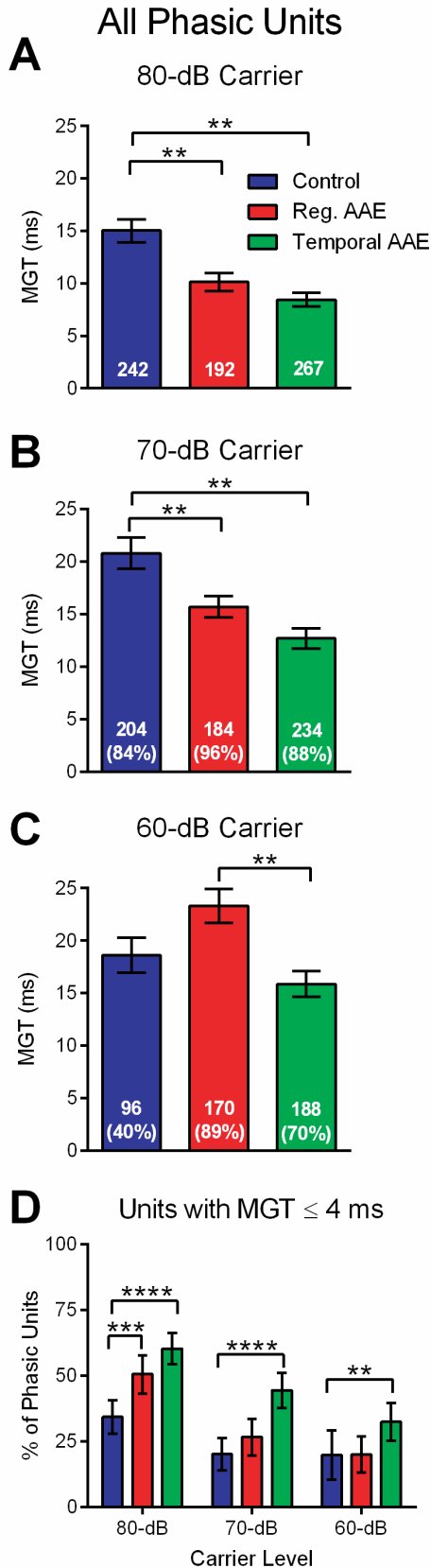
Figure 5. Exposure to both types of AAE improve mean gap thresholds in phasic units. Mean gap thresholds (MGTs) were computed across each group, for each noise carrier level (80, 70 & 60 dB). Total number of units in each group are shown at the bottom of the bar. **A**, At the 80-dB carrier level exposure to both types of AAE resulted in significantly shorter mean MGT (Control: 15.0 ± 1.1 ms, Reg. AAE: 10.1 ± 0.9, Temporal AAE: 8.4 ± 0.7 ms). **B,** At the 70-dB carrier level, exposure to both types of AAE again significantly shorten mean MGT, with greater improvement seen in the temporal AAE exposure group (Control: 20.8 ± 1.5 ms, Reg. AAE: 15.7 ± 1.0, Temporal AAE: 12.7 ± 1.0 ms). **C**, At the 60-dB carrier level the mean MGT from the temporal AAE group was significantly shorter than from the regular AAE group (15.9 ± 1.2 ms vs 23.3 ± 1.6 ms, $p < 0.01$). Additionally, the mean MGT for control mice was significantly shorter than from the regular AAE group (18.6 ± 1.7 ms vs 23.3 ± 1.6 ms) but the number of responsive units was much less. Sample size is shown inside the bar, with the percent equal to the percent of all phasic responsive units to an 80-dB carrier. **D**, A significantly greater number of phasic units had MGTs ≤ 4 msec in mice exposed to temporal AAE, followed by those exposed to regular AAE, for all carrier levels, 80, 70 and 60 dB SPL (** denotes p<0.05, ***=p<0.001, ****=p<0.0001 by Fischer exact test). The fraction of phasic units is displayed with $\pm 95\%$ CI.

359   Gap detection is more challenging in background noise, and may also be a key marker of

360   speech recognition difficulties in background noise [39, 40]. To determine whether this measure of

361   temporal acuity improves following AAE exposure, only units that responded to gap stimuli

362   (MGTs $\leq$ 96 ms) presented in continuous background noise (CBN) were included in the analysis

363   (see Table 1 and Figure 6A, B). This subpopulation also showed improvement in MGTs for gap

364   stimuli presented in quiet after either AAE exposure (One-way ANOVA: F = 18.16, p < 0.001;

365   post-hoc regular and temporal AAE MGT < control, p < 0.001; compare Figure 6A with Figure

366   5A). A one-way ANOVA also showed a significant effect of Exposure on MGTs when stimuli

367   were delivered in the presence of +6 dB SNR continuous background noise (F = 5.39, p = 0.005;

368   Figure 6B). Exposure to temporal AAE significantly shortened MGTs compared to controls (12.7

369    $\pm$ 1.0 ms vs. 17.9 $\pm$ 1.2 ms, p < 0.01), while exposure to regular AAE only trended towards shorter

370   MGTs (14.6 $\pm$ 1.2 ms vs. 17.9 $\pm$ 1.2 ms, p > 0.05). These data indicate that early temporal AAE

371   exposure improves gap detection in the presence of background noise for phasic units.
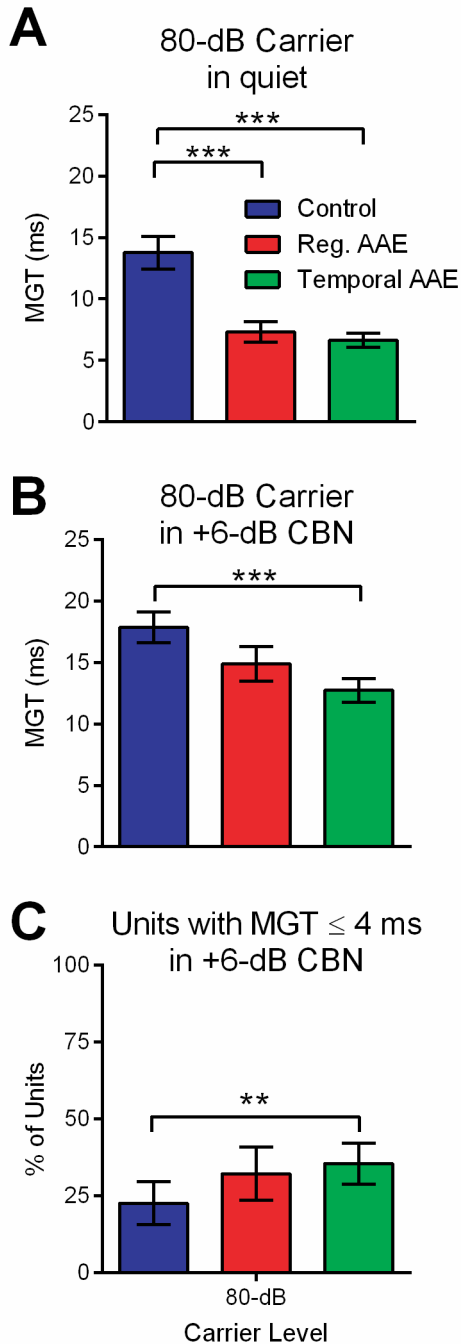
Figure 6. Exposure to temporal AAE preserves gap thresholds in the presence of continuous background noise (CBN). Only a subset of phasic units was responsive in background noise. **A**, Shown are the MGTs measured in response to an 80-dB carrier without CBN from only the units responsive in background noise. These results match those seen in Figure 5A where at 80-dB both types of AAE exposure resulted in significantly shorter MGT. **B**, The same units as in A, measured in response to an 80-dB carrier with +6 dB SNR CBN (background noise at 74 dB). Only exposure to temporal AAE resulted in significantly shorter gap thresholds compared to controls (12.7 ± 1.0 ms vs 17.9 ± 1.2 ms, $p < 0.01$). Sample size is given in each bar, with percent shown as a percent of all phasic responders at 80-dB. **C**, The percent of all responsive phasic units with MGTs ≤ 4 ms (sensitive responders) was increased in the temporal AAE group at all levels (** denotes p<0.05 by Fischer exact test. The error bars show fraction of phasic units with $\pm 95\%$ CI.

395   As our previous work suggests that behavioral gap thresholds are more strongly influenced

396   by midbrain units with the shortest gap thresholds [17], we computed the percent of responding units

397   with gap thresholds ≤ 4 ms (Figure 6C). All phasic units with MGT ≤ 96 ms (those shown in

398   Figure 5A-C) were included for this analysis. Temporal AAE-exposed mice had the greatest

399   percent of phasic units that had short gap thresholds, regardless of carrier intensity. Thus, early

400   temporal AAE strengthens encoding of short gap durations.

401

402   **Discussion**

403   In the present study, we have shown that exposure to a temporally-complex broadband

404   AAE can modulate multiple aspects of peripheral and central auditory function in a mouse model

405   of severe congenital SNHL. Early exposure to a novel, temporally-enriched broadband noise

406   stimulus, starting before hearing onset, improved ABR thresholds, wave I ABR amplitudes and

407   DPOAE amplitudes relative to normally-raised mice. The frequency range that showed the most

408   improvement in cochlear function was within the region of maximal energy for the AAE exposure

409   spectrum. Improvements in sensitivity and spectral encoding were also present in the CAS.

410   Recordings in the auditory midbrain showed lower neural thresholds to sounds and better

411   representation of higher frequencies following an enriched versus control environment.

412   Importantly, neural gap detection improved in both quiet and background noise, indicating

413   increased temporal processing acuity after this novel AAE intervention. Together these findings

414   suggest that early exposure to temporally-modulated broadband noise stimuli can restrict the

415   negative consequences of SNHL on peripheral function, and spectral and static temporal

416   processing in the CAS.

417   Sensorineural damage in the cochlea decreases sensitivity and distorts auditory input from

418   the periphery [41]. Consequent to this peripheral damage, a number of structural and

419   neurophysiological changes occur in brainstem, midbrain, and cortical auditory brain regions [42-51].

420   Altered central auditory processing associated with SNHL, such high thresholds, loss of frequency

421   representation, and broader tuning curves[26, 52], may impair auditory signal detection and

422   differentiation. Additionally, very early SNHL, experienced in DBA mice and infants with

423   congenital SNHL, may interact with normal developmental timelines for central auditory

424   processing to further impair sound processing beyond the direct consequences of SNHL. In normal

425   hearing humans and rodents, temporal processing acuity, as measured by gap thresholds, improves

426   during early development [53, 54]. Here we find deficits in neural correlates of gap detection in the

427   auditory midbrain in 1-month old DBA mice relative to normal hearing strains [17, 37]. Thus, while

428   SNHL in adulthood does not have profound effects on temporal processing in the auditory

429   midbrain [37], the current findings suggest that SNHL during development may strongly impact both

430   spectral and temporal aspects of central auditory processing.

431   Previous work indicates that in young mice with progressive, congenital SNHL, early

432   exposure to broadband AAE can preserve hearing sensitivity and limit hair cell loss [26-28, 30, 55]. This

433   also appears to limit concomitant reorganization in the CAS, limiting the loss of neurons in the

434   cochlear nucleus and sensitivity to high frequency sounds in the IC [28, 30]. Here we expand this

435   characterization of central auditory processing after early temporally modulated broadband AAE

436   exposure, finding that intervention can preserve both spectral and temporal acuity in the auditory

437   midbrain. After AAE intervention with our novel temporally complex AAE, and to a lesser extent

438   with a less complex broadband AAE, neural sound processing in the IC exhibited lower response

439   thresholds, greater high frequency encoding, more narrow frequency response areas, and better

440 gap encoding and detection. Others have reported similar CAS plasticity following more general

441 environmental enrichment with an auditory component. Recordings in the auditory cortex (AC)

442 showed improved neural temporal response properties, increased spectral and temporal selectivity,

443 and more narrow neural response fields [56, 57]. The current findings continue to support the idea that

444 both peripheral and central auditory processing can be modulated by enriched environments.

445 The plasticity of the CAS is remarkable across mammalian species. Like rodents, humans

446 exposed to various types of passive AAE that alter sound input to the ear also undergo profound

447 central auditory and perceptual changes resulting from neural plasticity. The effects of AAE on

448 central auditory function, particularly in the face of hearing loss, may arise, at least in part, from

449 homeostatic mechanisms that maintain neural activity (Turrigiano, 1999). Consistent with this

450 idea, when sound input is attenuated via deprivation (i.e., via temporary earplug, or conductive

451 hearing loss), the reduced peripheral input leads to increased central activity (see [58]). Subsequent

452 (or concurrent) exposure to passive AAE is predicted to stabilize peripheral excitatory drive and

453 preserve input to the CAS. By stabilizing the mean level of neural activity, AAE is predicted to

454 counteract hearing loss-related increases in central gain, improving coding efficiency, and

455 maintaining an optimal input-dependent dynamic range [59]. Indeed, perceptual changes in humans

456 are observed following AAE that are consistent with normalized gain, including altered loudness

457 perception [60, 61], and finer intensity resolution [62]. AAE may also help to maintain or expand sound

458 representation in the face of deteriorating peripheral input through standard experience-

459 dependent plasticity mechanisms. Humans show improved temporal coding following AAE [63],

460 which could arise from improved sound representation. In rodents, AAE can lead to

461 reorganization in primary and non-primary auditory cortex as reflected in narrower response

462 fields, improved temporal response properties, and increased spectral and temporal selectivity of

463   neurons [57, 64].

464   Intriguingly, while both types of passive AAE employed in this study improved auditory

465   sensitivity and spectral sound processing, our novel, temporally complex broadband AAE had a

466   stronger positive influence on temporal processing acuity. This was particularly true for

467   improvements in temporal processing with background noise. Thus, the benefits of early AAE

468   exposure may be related to characteristics of the sound presented. Previous work showed that in

469   young DBA mice, treatment with broadband AAE improved behavioral and neurophysiological

470   measures of tonal thresholds [26]. In the present study, a similar, but temporally more complex,

471   broadband AAE stimulus, improved both spectral and temporal sound processing. Band-limited

472   AAE also slowed the progression of SNHL in the 16 to 32 kHz range, but did not ameliorate a loss

473   of sensitivity at lower frequencies [55]. Likewise, the effect of AAE exposure is also shaped by

474   auditory function and timing of the intervention. In mature auditory systems, or with normal

475   hearing, some types of AAE exposure may instead lead to the suppression of sound sensitivity [30,

476   65]. Recently it was observed that young adult CBA mice exposed to 75 dB SPL AAE were found

477   to display functional evidence of cochlear synaptopathy [66]. Likewise, in adult cats with normal

478   hearing, tonal or band-limited AAE exposure profoundly suppressed AC activity in the frequency

479   range of the exposure [67-70].

480   Though the precise mechanism is unknown, early broadband AAE has a positive impact

481   on cochlear health across a limited tonotopic range depending on the spectral composition of the

482   AAE [28]. Improved cochlear function in turn supports lower thresholds and maintains frequency

483   representation in the CAS. However, this study re-affirms that broadband AAE exposure invokes

484   further adaptive neural plasticity in the auditory midbrain. Sharper IC neural tuning curves indicate

485   that lateral inhibition is enhanced in the CAS following auditory enrichment. Moreover,

486   temporally complex AAE may not only strengthen inhibition, but also improve the timing of

487   inhibition. Timed inhibition is key for shaping sound offset responses that subserve gap detection

488   [71-74]. Since more spectrally complex signals evoke stronger sound offset responses, the broadband

489   nature of the stimulus might be important, and may even be improved upon with a variable spectral

490   component [75]. The possibility that temporal encoding can be altered by auditory experience was

491   first confirmed by Kilgard and Merzenich [43], who demonstrated that the ability of AC neurons to

492   follow high frequency sound stimuli can be improved if high frequency sound stimuli are paired

493   with electrical stimulation of the nucleus basalis. The improvement in neural encoding and

494   detection of gaps in noise in the current study shows that even passive sound exposure may shape

495   temporal acuity, though it seems unlikely that this occurs through pathways involving the nucleus

496   basalis.

497   Overall, the current findings suggest that temporally-complex AAE interventions may

498   provide functional benefits in individuals with SNHL, especially newborns diagnosed with hearing

499   loss. The improved neural encoding of short gap durations in the IC is likely to support functional

500   improvements in gap detection, as these measures are strongly associated [17]. In turn, improved gap

501   detection, particularly in noise, counteracts an aspect of hidden hearing loss that impairs speech

502   perception in daily life [12]. Though not specific to the temporally complex AAE intervention, better

503   frequency representation and sharper spectral tuning that occurs after AAE may also bolster

504   auditory signal detection and differentiation. These findings support the possibility that AAE may

505   be targeted, based on the properties of the AAE as well as the listener, to better improve hearing

506   deficits. The ability of our novel, temporally complex broadband AAE exposure to improve neural

507   correlates of SNHL provides direct bench-to-bedside promise for treating congenital SNHL.

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