Auditory steady-state responses: multiplexed amplitude modulation frequencies to reduce recording time

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Abstract

This study investigated the efficiency of a multiplexed amplitude-modulated (AM) stimulus in eliciting auditory steady-state responses (ASSR). The multiplexed AM stimulus was created by simultaneously modulating speech-shaped noise with three frequencies: 3.1, 40.1, and 102.1 Hz. For comparison, a single AM stimulus was created for each of these frequencies, resulting in three single AM conditions and one multiplex AM condition. Twenty-two bilaterally normal-hearing participants (18 female) listened for 8 minutes to each type of stimuli. The analysis compared the signal-to-noise ratios (SNRs) of the evoked responses to the single and multiplexed conditions. The results indicated that the SNRs evoked by the single AM conditions were, on average, 1.32 SNR dB higher than those evoked by the multiplexed AM condition, but this difference was insignificant. Only when considering various stimulus durations ranging from 1 to 8 min did the single conditions evoke a significant 0.55 SNR dB higher than the multiplexed SNR dB. However, both single and multiplexed conditions evoked significant ASSR within subjects. On average, the multiplexed AM stimulus requires 9 minutes to elicit a significant response across all three frequencies, while the single AM stimuli require 14 minutes. These findings show that the multiplexed AM stimulus is a promising method to reduce the recording time to measure the effect of modulation frequency while simultaneously obtaining information from various neural generators.

Terms: Auditory steady-state response (ASSR), multiplexed amplitude-modulation

1. Introduction

The auditory steady-state response (ASSR) is a commonly used objective measure of the auditory system (ASSR; Picton & Burkard, 2012; Picton et al., 2003). During the test, electrodes are placed on the scalp to record the electrical signal generated by the brain in response to an auditory stimulus, requiring minimal subject participation. The ASSR uses an amplitude-modulated (AM) carrier wave at a specific frequency. Listening to this stimulus evokes sustained neural activity that is phase-locked to the stimulus's modulation frequency. The response can be elegantly and fully automatically detected by signal processing and statistical methods determining when the modulation frequency is significantly present in the brain signals (Van Dun et al., 2008). Responses to slow AM frequencies ($<$ 20 Hz) originate primarily from cortical areas (Alaerts et al., 2009; Farahani et al., 2020; Herdman et al., 2002; Y. Wang et al., 2012), while responses to faster AM frequencies ($>$ 80-100 Hz) originate primarily from subcortical areas such as the brainstem (Bidelman, 2015; Farahani et al., 2020; Herdman et al., 2002; Luke et al., 2017).

Recording ASSRs can be time-consuming. Research aiming to provide an efficient ASSR often focuses on using a multiple ASSR stimulus, an auditory stimulus where multiple carrier waves ranging from 0.5 to 8 kHz are used. Each carrier wave is linked to an AM frequency, and all pairs are presented simultaneously rather than sequentially, which saves time during the session and improves efficiency (Hatton & Stapells, 2010; Luts & Wouters, 2004). However, the AM frequencies are usually selected from a small range, such as 30 to 50 Hz (e.g., Gransier et al., 2017) or 80 to 110 Hz (e.g., Luts et al., 2006), in order to target specific neural generators. This approach has a drawback because it only tests a limited number of neural generators in the auditory pathway and not all of them.

This study aims to create a new type of ASSR that can efficiently gather information from various neural generators of the auditory system. The current standard method requires the subject to listen to a series of ASSR stimuli, where each stimulus is amplitude modulated by a different frequency. These single ASSR stimuli each target a different neural generator in the auditory pathway. We propose a novel ASSR stimulus called the "multiplexed AM stimulus". This stimulus consists of a single carrier wave, which is a speech-shaped noise. The carrier wave is simultaneously amplitude modulated by multiple frequencies selected from a broad frequency range ($<$ 10 Hz to $>$ 100 Hz). This broader range allows the stimulus to simultaneously target multiple neural generators in the auditory pathways, such as the brainstem, midbrain and auditory cortex. In summary, the multiplexed stimulus may help us efficiently obtain information from various neural generators by reducing the recording time, potentially reducing the impact of tiredness and loss of attention.

2. Materials and methods

2.1. Subjects

We recruited 22 bilaterally normal-hearing subjects (4 male and 18 female) with a mean age of 22.7 ± 2.6 years. All subjects participated voluntarily and signed an informed consent. The Ethics Committee Research UZ / KU Leuven approved the experiment (S57102).

2.2. Experimental procedure

At the start of the experiment, subjects were placed in a soundproof Faraday shielded booth, and ASSR stimuli were binaurally presented via ER-3A insert earphones (Etymotic Research

Inc, IL, USA) to each subject. The earphones were encased in an electrically grounded box. Subjects partook in three single amplitude-modulated conditions and one multiplexed AM condition, lasting around 32 minutes. During each condition, subjects listened for eight minutes to an amplitude-modulated carrier presented using the APEX software (Francart et al., 2008). The order of these conditions was randomized for each subject. The stimulus was presented using an RME Fireface UC soundcard (Haimhausen, Bayern, Germany) at 80 dBA.

2.3. Stimulus construction

For the stimulus carrier, we used speech-shaped noise based on a male speaker. The carrier was 100% modulated in the single AM conditions by either a 3.1 Hz, a 40.1 Hz, or a 102.1 Hz sinusoidal modulator. We chose these frequencies based on the neural generators they target. Specifically, the 3.1 Hz AM frequency targets the cortical areas, the 102.1 Hz AM frequency targets the brainstem, and the 40.1 Hz AM frequency targets the cortical areas, the brainstem, and the thalamus. For the multiplexed AM condition, the carrier was simultaneously 100% modulated by all AM frequencies, i.e., a multiplexed modulator; see Equation 1, where m, the multiplexed modulator, is the element-wise multiplication of the 3.1 Hz sinusoidal modulator (m_3), the 40.1 Hz sinusoidal modulator (m_{40}), and the 102.1 Hz sinusoidal modulator (m_{102}). From here on, we will refer to the stimuli and evoked potentials as 3 Hz, 40 Hz, and 102 Hz for convenience.

$$m = m_3 \odot m_{40} \odot m_{102} \tag{1}$$

Figure 1A-D shows the resulting modulators for single and multiplexed conditions. This figure shows that when we multiply single sinusoidal modulators to create a multiplexed modulator, an amplitude reduction is observed in the resulting signal. To quantify this amplitude reduction for each frequency component, we calculated the Amplitude Attenuation Index(AAI); see Equation 2.

$$AAI_f = \frac{\int_0^{2\pi} |m(t)| dt}{\int_0^{2\pi} |m_f(t)| dt} \tag{2}$$

In the equation, the multiplexed modulator is denoted as $m(t)$, where t stands for time, and $m_f(t)$ represents one of the single sinusoidal modulators used to create the multiplexed modulator. The AAI_f value indicates the amplitude attenuation experienced by a specific frequency component f within the multiplexed modulator. An AAI_f of 1 signifies that the frequency component in the multiplexed modulator did not undergo any amplitude attenuation. Conversely, an AAI_f of 0 suggests a complete amplitude attenuation of the frequency component within the multiplexed modulator. The AAI for each frequency component in the multiplexed modulator is shown in Figure 1E. Since the frequencies 3 Hz, 40 Hz, and 102 Hz have no common denominator, the AAI varies across different periods of the multiplexed modulator. However, on average, the AAI is 0.4 for each frequency component, which indicates that the amplitude of each frequency component is only 0.4 of its original amplitude.

2.4. EEG acquisition

Subjects' brain activity was recorded using electroencephalography (EEG) with a 64-channel BioSemi ActiveTwo system (BioSemi, Amsterdam, Netherlands) with Ag/AgCl electrodes mounted on a cap according to the 10-20 system of electrode

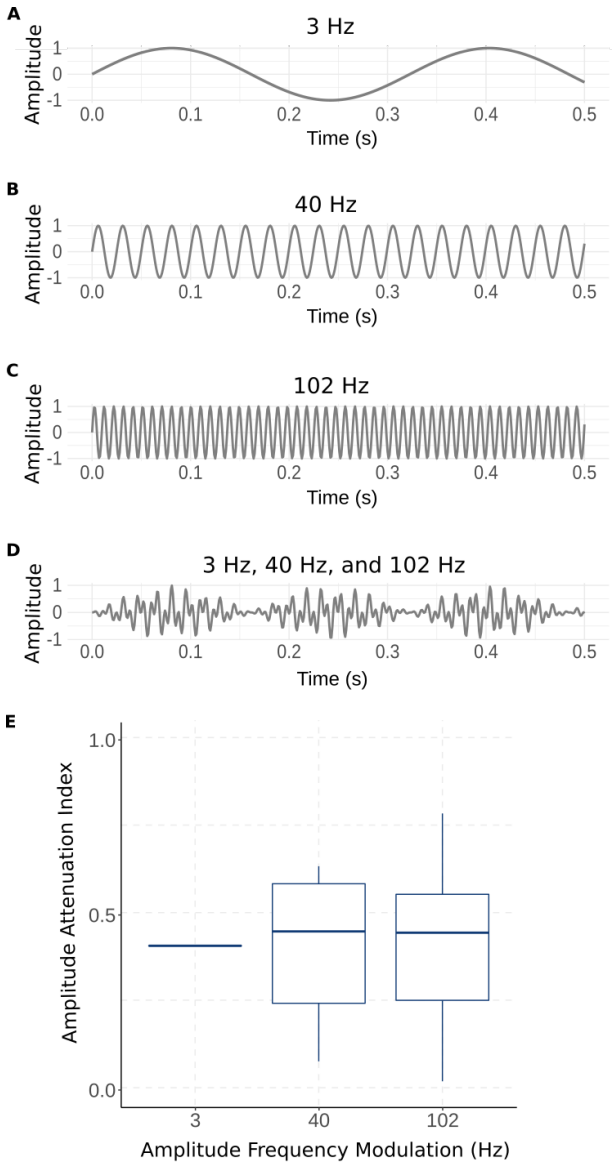

Figure 1: *Amplitude modulated frequencies with their respective envelope (A) AM = 3 Hz (B) AM = 40 Hz (C) AM = 102 Hz (D) AM = 3, 40, 102 Hz. (E) The AAI for each frequency of the multiplexed modulator.*

placement (Oostenveld & Praamstra, 2001). The EEG data were acquired using the ActiView software at an 8192 Hz sampling rate.

2.5. Signal preprocessing

The electroencephalography (EEG) data preprocessing was performed offline in the MATLAB environment ("MATLAB", 2021).

2.5.1. Signal preprocessing

We extracted the amplitude of each frequency in the EEG signal by resampling the raw EEG to a sampling rate of 1024 Hz and applying a 20th-order infinite impulse response comb-notch filter to remove the 50 Hz powerline and its harmonics. Bad channels were identified using the root mean (RMS) method, which involves calculating the RMS value for each channel and comparing it to the threshold of $3\times$ the median RMS across channels. Channels with RMS values above this threshold are considered bad channels and were then interpolated using adjacent channels. The signal was then filtered using a 4th-order Butterworth high-pass zero-phase filter with a cutoff frequency of 1 Hz to remove DC and drift components. A multi-channel Wiener filter (Somers et al., 2018) was applied to remove eye-blinking artifacts, and the signal was re-referenced to the Cz electrode.

2.5.2. Signal preprocessing for the SNR calculation

To extract the SNR of each frequency in the EEG signal, the same bad channels detected during the amplitude preprocessing were interpolated. The signal was re-referenced to the Cz electrode and divided into epochs based on the stimulus frequency. Since the 3 Hz, 40 Hz, and 102 Hz do not have a common denominator to align their phases; we opted to make slight variations in epoch timing to ensure we have an integer number of cycles such that no phase drift could occur. For the 3.1 Hz stimulus, the signal was divided into 0.9677-second epochs, resulting in 7928 samples per epoch. For both the 40.1 Hz and 102.1 Hz stimuli, the signal was divided into 0.9989-second epochs, resulting in 8184 samples per epoch. After dividing the signal into epochs, a quality control step was performed to identify and discard bad epochs. It was discarded if an epoch's peak-to-peak value was higher than six times the median of the peak-to-peak values obtained across all the channels for each given epoch.

2.5.3. Electrode selection

The SNR was first normalized within subjects and then averaged per modulation frequency using the min-max function (see Figure 2). Across modulation frequencies, the highest normalized SNR was observed for the P10, and P9 electrodes, located in the parietal-occipital region of the brain (see Figure 2B), and thus were chosen as our electrode selection. All results are averaged across P9 and P10.

2.6. Outcome measures and statistics

2.6.1. Signal-to-noise ratio

Each frequency bin's SNR was determined using Hotelling's T^2 statistic (Hotelling, 1992), a statistical approach that generalizes the Student's t-statistic to a multivariate dataset. In our study, we used Hotelling's T^2 statistic to compare the mean of the modulated frequency bin to the bin's variability across epochs, allowing for the detection of synchronized activity in

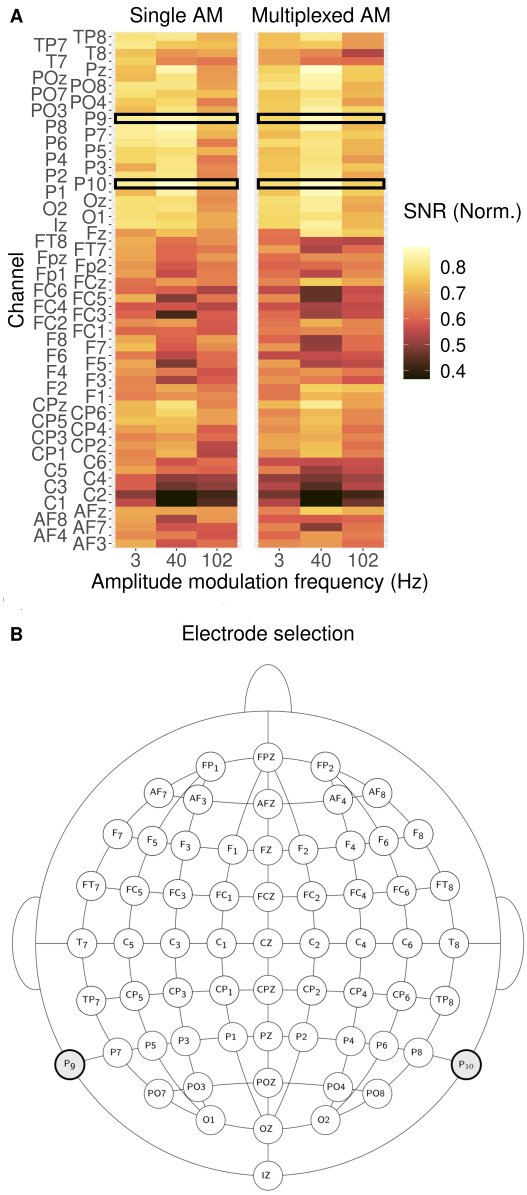

Figure 2: *(A) The average signal-to-noise ratio (SNR) normalized per condition across participants. Electrode P10 and P9 show the highest SNR across modulation frequencies. (B) The electrode selection is shown on a 10-20 topographical electrode map.*

the EEG signal. We applied the Hotelling T^2 statistic to each EEG channel to determine if the synchronized activity evoked by the ASSR stimuli was significantly different from the non-synchronized neural background activity. Negative SNR values are not meaningful, as SNR values are expected to be positive. Therefore, any negative SNR value was set to zero.

2.6.2. Linear mixed effect model: single vs. multiplexed

We constructed a linear mixed-effect (LME) model using R (R Core Team, 2020) and the lme4 package (Bates et al., 2015) to investigate the relationship between listening to a multiplexed AM stimulus, listening to a single AM stimulus, and the various

AM frequencies on the SNR of the evoked response. Initially, we fit a linear mixed-effects (LME) model to our data, including all predictor variables and their interactions. However, the interaction effects between the AM frequencies and the ASSR condition(single vs. multiplexed) were found to be non-significant and were subsequently removed from the model, see Equation 3.

$$SNR(dB) = \alpha_j + \beta_1(f_{40}) + \beta_2(f_{102}) + \beta_3(condition) + \epsilon_j \tag{3}$$

The model includes three categorical variables: $f40$ (representing a 40 Hz AM frequency), $f102$ (representing a 102 Hz AM frequency), and $condition$ (representing the ASSR condition, with 0 indicating single and 1 indicating multiplexed). These variables are considered fixed effects in the model. The 3 Hz AM frequency is also included in the model's intercept. Interaction terms are added between the AM frequencies and the conditions. As random effects, the intercepts for the subjects α_j are included to account for differences between subjects, and the residual error term ϵ_j represents the variability that the model does not explain. Although the residuals of our linear-mixed effect model do not follow a normal distribution, resulting in less model power, we still proceeded with the planned analysis. To interpret the LME model, we conducted post-hoc tests using the emmeans package in R (Lenth, 2023) with Tukey's multiple comparison test (Keselman & Rogan, 1977).

2.6.3. Linear mixed effect model: SNR over ASSR duration

To investigate at which amount of data the group-level response is significant with 95% confidence, we applied Hotelling's T^2 statistic to varying amounts of data for each modulation frequency, for each ASSR condition, and for each participant. The data was sampled in one-minute intervals, ranging from one to eight minutes. We chose to fit a square root linear mixed-effects (LME) model to this data as a previous study has shown that the square root function best describes signal-to-noise ratio over increasing amounts of data (Elberling & Don, 1984). We initially fitted a linear mixed-effects (LME) model to our data, including all predictor variables and their interactions. However, as noted, like the other LME model, the interaction effects between the AM frequencies and conditions were found to be non-significant and were subsequently removed from the model, as shown in Equation 4. Similar to our previous model, the residuals of our second linear-mixed effect model do not follow a normal distribution, which could result in reduced model power. Nevertheless, we again proceeded with the post-hoc tests and analysis as planned.

$$SNR(dB) = \alpha_j + \beta_1(f_{40}) + \beta_2(f_{102}) + \beta_3(condition) + \beta_4(\sqrt{duration}) + \beta_5(f_{40} * \sqrt{duration}) + \beta_6(f_{102} * \sqrt{duration}) + \epsilon_j \tag{4}$$

3. Results

3.1. Median SNR above significance threshold for each modulation frequency

The SNR of the evoked responses was calculated for each participant in the study using the eight-minute EEG recording. At eight minutes of data, the F-value that matches a significance threshold with alpha equal to 0.05 is 3.0145, which translates to 4.78 SNR dB (Dobie & Wilson, 1996). Across participants, the median SNR for single and multiplexed ASSR conditions was above 4.78 SNR dB, see Figure 3A. For the 40 Hz AM, all evoked responses are above the significance threshold. For the 3 Hz AM, 68% (15 out of 22) of the participants displayed significant responses to the multiplexed AM stimulus, while 81% (18 out of 22) displayed significant responses for the single AM stimulus. A similar pattern was observed for the 102 Hz AM, with 77% (17 out of 22) of participants displaying significant responses to the multiplexed AM stimulus and 86% (19 out of 22) displaying significant responses to the single AM stimulus. These results indicate that the single and multiplexed AM stimuli elicit significant synchronized activity in the EEG signal.

Further support for these findings is provided by analyzing the frequency spectrum, as illustrated in Figure 4. For the demonstration, two subjects were chosen. Subject S02 shows a clear evoked ASSR at the AM frequencies for the multiplexed and the single AM stimuli. In contrast, the responses of subject S11 have a lower amplitude, and only the peak at 40 Hz can be discerned. The frequency spectrums across subjects were largely comparable between single and multiplexed AM stimulation.

3.2. No significant difference in SNR for multiplexed vs. single AM stimuli

We investigated the potential difference in SNRs between the ASSRs evoked by a single AM stimulus and those evoked by a multiplexed AM stimulus. Our LME model, which had an R^2 of 0.61, found that the 40 Hz ($t[110] = 10.858, p < .001$) and 102 Hz ($t[110] = 3.833, p < .001$) categorical variables, as well as the intercept ($t[53.35] = 8.265, p < .001$), had a significant effect, whereas the multiplexed fixed effect did not significantly affect the SNR value ($t[110] = -1.740, p = 0.084598$). To ease the interpretations, we also conducted post hoc tests on the LME model; see Table 1. The pairwise comparison between the estimated marginal means of the single and multiplexed stimuli showed no significant difference in the SNR values ($difference = 1.32SNRdB, t[113] = 1.716, p - value = 0.5239$), indicating that using a multiplexed AM stimulus does not significantly change the SNR values compared to using a single AM stimulus with 95% confidence interval (CI) [-0.2, 2.84].

Multiplexed	AM frequency	Mean (SNR)	CI 2.5%	CI 97.5%
0	3	8.79	6.62	10.96
1	3	7.47	5.30	9.64
0	40	18.87	16.70	21.04
1	40	17.55	15.38	19.72
0	102	12.35	10.18	14.52
1	102	11.03	8.86	13.20

Table 1: *The table shows the post hoc estimates and the 95% confidence interval (CI) for the fitted LME model; see Equation 3. The estimates in the table represent the estimated marginal means while holding all other variables in the LME model constant.*

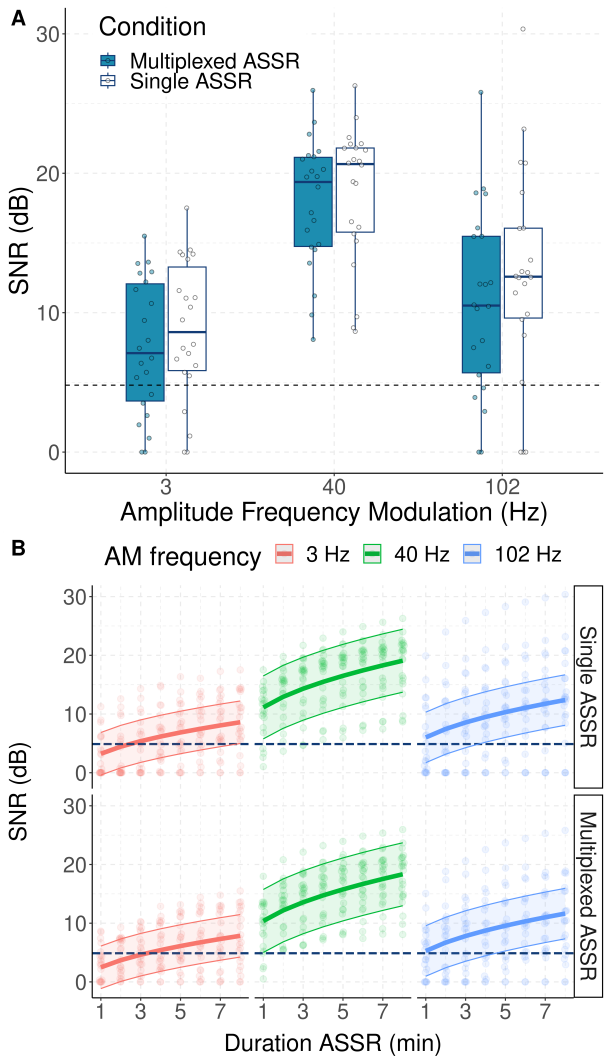

Figure 3: *(A) The boxplots compare the amplitudes evoked by the single stimuli against those evoked by the multiplexed stimulus. A dot in each condition represents each subject. (B) compares the SNRs evoked by the 3, 40, and 102 Hz AM frequencies for the single and multiplexed ASSR across stimuli duration. Each dot represents the computed SNR for each subject at each duration, while the bold lines show the average fit across subjects. The confidence interval is depicted between the two outer lines.*

3.3. Using a multiplexed AM stimulus reduces the recording time compared to using sequentially presented single AM stimuli

To complement our current findings, as depicted in Figure 3A, we sought to determine the minimum stimulus duration required to elicit a significant response for each type of auditory ASSR across all participants. Our fitted model, shown in Equation 4, had an R^2 value of 0.71 and revealed the following fixed effects to be significant: ASSR condition ($t[1034] = -3.317, p < .001$), $\sqrt{duration}$ ($t[1034] = 8.858, p < .001$), 40 AM frequency ($t[1034] = 6.522, p < .001$), and 102 AM frequency ($t[1034] = 2.245, p < .05$). We also found that the interaction effect between the 40 Hz and $\sqrt{duration}$ was significant ($t[1034] = 3.009, p < .01$). The post hoc test showed a 0.747 SNR dB ($CI = [0.3, 1.2]$) difference between the multiplexed and single ASSR. Overall, both ASSR conditions showed a similar evolution of SNR across time, as shown in Figure 3.

We then investigated the minimum stimulus duration required for each AM frequency to evoke a significant response with 95% confidence. Our results showed that for the 40 Hz AM frequency, only one minute of stimulus duration was needed for both ASSR conditions to elicit an average significant response. However, for the 102 Hz AM frequency, a longer stimulus duration of 5 minutes was required for the single ASSR and 6 minutes for the multiplexed AM stimulus. For the 3 Hz AM frequency, 8 minutes of stimulus duration was insufficient for the multiplexed ASSR. This finding suggests that approximately 9 minutes of stimulus duration is necessary to obtain a statistically significant response for the multiplexed ASSR, as the confidence interval nearly crosses the threshold of significance at the 8-minute mark. The critical difference between the multiplexed and single stimuli was that the multiplexed stimulus allowed for the presentation of all AM frequencies simultaneously, resulting in a shorter overall duration of 9 minutes to elicit a significant response. In contrast, for the sequentially presented single stimuli, the stimulus duration for each AM frequency must be added together, resulting in a total duration of 14 minutes. Our findings suggest that the multiplexed stimulus is more efficient and effective for eliciting significant ASSR responses across multiple AM frequencies.

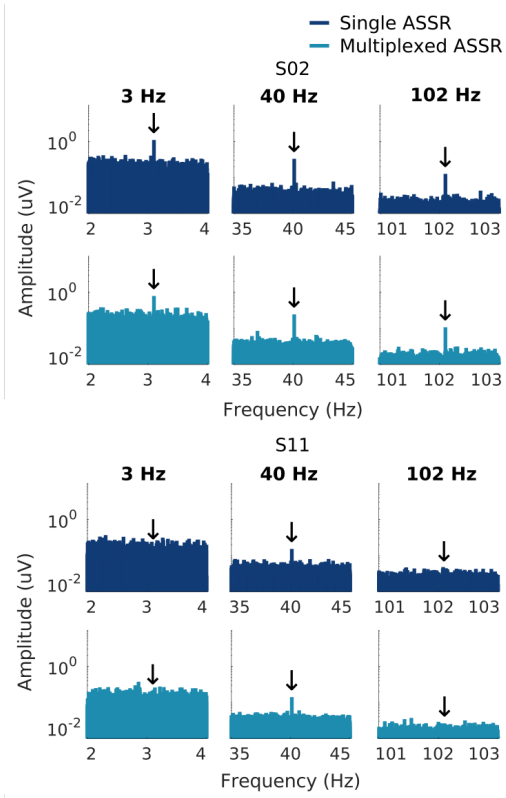

Figure 4: *The figure shows the amplitude across frequencies of the ASSR response for subjects S02 and S11, with arrows pointing to the amplitude-modulated frequencies of 3 Hz, 40 Hz, and 102 Hz.*

4. Discussion

This study compared the standard single AM stimulus, where each AM frequency is presented sequentially, to a novel approach called the multiplexed AM stimulus. The multiplexed AM stimulus is a single carrier wave modulated by multiple frequencies simultaneously, aiming to obtain information from various neural generators efficiently or simply measure the effect of modulation frequency. This approach contrasts with "multiple ASSRs," where multiple carrier frequencies are used simultaneously. This study is the first to examine the ASSR to multiple simultaneously presented AM frequencies, specifically 3 Hz, 40 Hz, and 102 Hz, on a single carrier.

We found no significant difference between the single and multiplexed conditions on the SNR of the evoked response. However, when we studied the effect of stimulus duration on the ASSR, where we tested durations ranging from one to eight minutes and calculated the SNR of the ASSR for each duration, we found that the single AM stimulus had a significantly 0.55 higher SNR dB than the multiplexed AM stimulus. This finding could be attributed to an increase in data and subsequently increased statistical power. Furthermore, the model's residuals exhibit left skewness, increasing the likelihood of type I errors. As a result, caution should be exercised in interpreting this significant difference as it may be a false positive result. If this finding is true, it would mean that considering a single frequency, single ASSRs are slightly more efficient. This result makes sense because the single sinusoidal modulators have been 100% amplitude modulated. When these modulators are combined into a multiplexed modulator, only 40.52% of its original amplitude is kept, and this attenuation is also inconsistent across periods.

In most applications, ASSRs determine whether a response was present, irrespective of the amplitude or SNR. Therefore, as long as the response to both stimuli is above the significance threshold, the differences in SNR between both stimuli are less relevant. Thus, we used a linear-mixed effect model to determine each stimulus's required duration to reach the SNR threshold. The multiplexed AM stimulus requires approximately 9 minutes of presentation time to evoke a significant response on all AM frequencies, while the sequentially presented single AM stimulus requires approximately 15 minutes. Therefore, using the multiplexed AM stimulus is a valid alternative to using the single AM stimuli due to the reduced recording time.

Similar to previous studies, the SNRs of the ASSR exhibit an inverted U-shaped pattern across AM frequencies, with the highest SNR values appearing at around 40 Hz (Gransier et al., 2021; Gransier et al., 2017). Previous studies have shown that the auditory system has the highest sensitivity to AM frequencies around 40 Hz and that this response originates from various neural generators (Farahani et al., 2017; Farahani et al., 2020; Herdman et al., 2002; Johnson et al., 1988; Reyes et al., 2004; Weisz & Lithari, 2017). Furthermore, the percentage of significant ASSRs evoked by multiplexed and single AM stimuli are comparable to those reported by Gransier et al. (2021), Gransier et al. (2017), with all ASSRs evoked by the 40 Hz stimuli being significant. Notably, we found a higher number of significant responses for both the 3 Hz and 102 Hz AM frequencies, which can be attributed to the diotic stimulation used in our study, in contrast to the monaural stimulation employed by Gransier et al. (2017). Moreover, the stimuli in our study were presented at a sound intensity of 80 dB instead of 70 dB, which can also affect the amplitude of the ASSRs (Ménard et al., 2008).

The main result of our study shows that using a multiplexed AM stimulus can reduce recording time, which may be more practical for subjects prone to tiredness, loss of attention, and low arousal. Another advantage of the multiplexed AM stimulus is the ability to present a wide range of AM frequencies simultaneously. This approach allows the stimulus to target multiple neural generators in the auditory pathway. High AM frequencies above 80 Hz tend to elicit more subcortical activity, primarily originating from the brainstem (Bidelman, 2015; Farahani et al., 2020; Herdman et al., 2002; Luke et al., 2017). In contrast, low AM frequencies below 20 Hz elicit more cortical activity, primarily originating from the auditory cortex (Alaerts et al., 2009; Farahani et al., 2020; Herdman et al., 2002; Y. Wang et al., 2012).

A limitation of the current multiplexed AM stimulus is that, while we suspect it targets the various neural generators, we cannot precisely determine where the ASSR originates because the stimulus lacks sufficient frequencies to perform an apparent latency analysis (Picton et al., 2003). Although, our current results show that there are no noticeable differences with the single ASSR responses. Moreover, there is no theoretical basis to suspect that the neural generators responsible for the multiplexed ASSR responses differ from those involved in the single ASSR responses.

Based on the study results, a potential avenue for future research would be to improve the multiplexed AM stimulus by increasing the number of AM frequencies included. This approach would allow for an analysis of the apparent latency, which can provide more information about the neural generators involved in the multiplexed ASSR and therefore provide greater insights into the integrity of the auditory pathway (L. Wang et al., 2021). Additionally, increasing the number of AM frequencies can help determine the maximum number of frequencies that can be effectively combined into a single stimulus. Another avenue might investigate multiplexed sweeps, where sweeps on lower frequency ranges are simultaneously combined with sweeps on higher frequency ranges; this might help to better capture ASSRs from each neural generator, as the specific AM frequency that neural generators respond best to can vary strongly across individuals (Gransier et al., 2021).

In conclusion, our study revealed that employing a multiplexed ASSR is more time-efficient than using a sequentially presented single ASSR.

5. Conflict of interest

The authors declare no conflict of interest.

6. Acknowledgements

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