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High-Fidelity Auditory Evoked Potential Recording System: Design and Clinical Application to Objective Tinnitus Diagnosis Research

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BY

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Abstract

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The auditory evoked potential (AEP) is an electrical activity of the auditory system following the auditory stimulus presentation. The AEP is broadly applied not only as a clinical use for the testing of hearing threshold or auditory neuropathy, but also as a research tool for the investigation of fundamental mechanisms in many neurodevelopmental disorders. For these purposes, essential requirements for the high-quality AEP recording are the low system noise and precise auditory stimulus presentation due to its very low amplitude and stimulus dependence. Additionally, flexible stimulus control and real-time AEP data processing are advantageous for the researches on customized auditory stimulus paradigms and novel signal processing algorithms.

The first objective of this thesis was to design the flexible, high-performance AEP recording system as the form of a single platform in order to increase the operational stability. The low noise analog front-end and power regulation circuits were developed for the inference minimization. The various auditory stimuli generated from the integrated circuit were accurately calibrated complying with the international standard. Moreover, the parallel loop structure of the software enabled real-time AEP data processing. The evaluation results indicated that the developed system can be used for the high-fidelity AEP recording in terms of the system noise level and stimulus accuracy. In the real auditory brainstem response (ABR) and auditory late response (ALR) recordings from human subjects for further validation, clear waveform morphologies were confirmed and they were reproducible in all subjects.

The second objective of this thesis was to apply the developed system to the real clinical research by utilizing its flexibility in the stimulus control and AEP data processing. Toward objective tinnitus diagnosis, the customized gap-prepluse inhibition (GPI) paradigm used in animal studies was modified in the context of human subjects with the ALR recording. In the first normative study with healthy normal-hearing subjects, the N1-P2 complex of the ALR best reflected the GPI in terms of the inhibition ratio and test-retest reliability. The minimum required number of stimuli repetitions for the stable GPI ratio was also found to shorten the test time. Using these practical findings, the discriminative stimulus condition

showing the effect of tinnitus presence was found in the second comparative study with tinnitus patients. Thus, this novel approach using the ALR with the GPI paradigm may hold a promise as an objective measure of tinnitus in humans.

Keywords: Auditory evoked potential, Low noise system design, Tinnitus, Objective diagnosis, Gap-prepulse inhibition, N1-P2 complex
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Chapter 1. Introduction

1.1. Auditory Evoked Potential (AEP)

The auditory evoked potential (AEP) is an electrical activity of the auditory system from the ear to the brain following the onset of the auditory stimulus (1). The ionic current flow originated in the cochlea of the inner ear is sequentially transferred to the cerebral cortex through the ascending auditory pathway. The potential field, which is the distribution of this transmembrane ionic current flow in the extracellular, is the source of the voltage potential detected on the body surface. In general, the AEP is noninvasively recorded by surface electrodes located on the scalp and around the ear. The AEP is usually categorized by the latency and anatomical location of the auditory system generating the potential (2). First, the electrocochleogram (ECochG) reflects the compound evoked response of the cochlea and eighth auditory cranial nerve within the first 2 or 3 ms after the auditory stimuli. The subsequent response reflecting the auditory brainstem activity within about 10 ms is the auditory brainstem response (ABR). The auditory middle-latency response (AMLR) observed from about 12 to 50 ms reflects the neural activity originating from the thalamocortical auditory pathways. Finally, the response recorded from 50 to 600 ms reflecting the complex auditory cortical processing involved in the auditory and frontal cortex is the auditory late response (ALR). The number of involved neurons and the distance between the source and the electrode affect the AEP amplitude. While the ALR

generated in higher brain regions involving millions of neurons shows the amplitude range between roughly 1 and 10 μV, the ECochG and ABR generated by fewer neurons are recorded in sub-microvolt (0.1 to 0.5 μV). These amplitudes are rather very low when comparing to other surface-recorded biopotentials such as the spontaneous electroencephalogram (EEG) recorded in tens of microvolt or electrocardiogram (ECG) recorded in millivolts (3).

In general, typical positive or negative peak components are shown in the AEP waveforms. The ABR waveform typically consists of major five positive peaks labeled as the wave I to V. The AMLR waveform shows several positive and negative peak components labeled as the N_0, P_0, Na, Pa, Nb, and Pb in a sequential order. The last Pb component observed around the 50 ms latency sometimes is also referred to the P50 or the P1, the first peak of the ALR waveform. Subsequent major peaks labeled as the N1, P2, and N2 are typically shown in the ALR waveform. The latency and amplitude of peak components are affected by the stimulus type and intensity as well as physiological and psychological factors. Thus, the peak characteristics of the AEP waveforms have been extensively used in clinic as well as research studies (1). For example, typical ABR peak components are used as the objective hearing screening of newborn infants in clinic and the auditory threshold estimation of animals in research studies. The AMLR peak components have been applied to diagnose neuropathological disorders such as a tumor in the auditory pathway (4-6). The ALR peak components have

been investigated for the assessment of the higher level auditory processing such as auditory discrimination or speech perception (7-9). Especially, the amplitude variation of the P1, N1, or P2 components under various stimulus paradigms such as the prepulse inhibition or odd ball paradigm has been intensively analyzed in research studies of pharmacology (10-12) and neuropsychiatric disorders such as schizophrenia, depression, or autism (13-18).

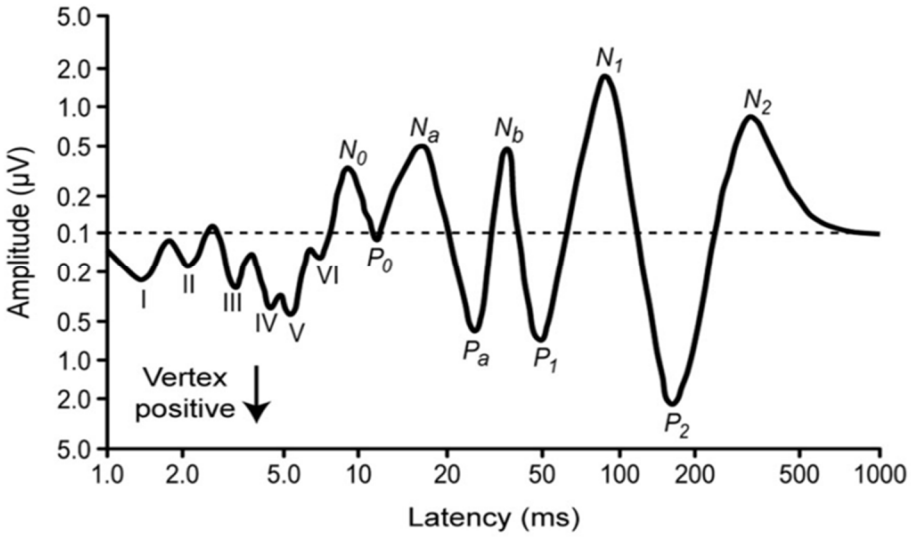

Figure 1.1 Typical waveform of the auditory evoked potential (adapted from (2)).

1.2. System Requirements

In the AEP recording, very low AEP signal is buried within other brain activities (i.e. spontaneous EEG) and can be easily degraded by the eye blink artifact or system electrical noise. The AEP recording system requires high signal amplification and synchronized signal averaging by stimulus repetitions. For the ABR, especially, over 100,000 times amplification and over 1,000 stimulus repetitions are recommended (1). Thus, low noise performance is very essential for the AEP recording system because large system noise could cause the signal saturation within amplification stages and increase the number of stimulus repetitions, which lengthen the recording time. The signal amplification should be accompanied by appropriate analog filtering to avoid the signal saturation. The gain and cut-off frequency of the analog front-end in the AEP recording system are determined by the target signal. For example, the frequency bandwidth that can cover up to the 3,000 Hz is required for the ABR recording while the narrower frequency bandwidth with the 1 to 100 Hz is recommended for the ALR recording (1). Additional digital filters can applied for further smoothing depending on recording purposes. Thus, flexible control of recording parameters in the AEP recording system is required for various clinical and research applications. Moreover, flexible raw data processing of the AEP waveforms is necessary for the clinical researches because further signal processing algorithms besides the analysis of the amplitude or latency of main peak components are frequently utilized to find the evidence proving the research hypothesis.

In addition, the stimulus presentation in the AEP recording system should be accurately controlled because the AEP waveform morphology is varied depending on the intensity and frequency of the auditory stimulus. Apparently, the ABR recording for the hearing screening in clinic should be performed with the accurate intensity of the sound output. Moreover, clinical researches using the ALR try to exclude the effect of the subjects' hearing ability by presenting the accurate intensity of auditory stimulus in hearing sensation level. The flexibility of the stimulus generation is also required in the AEP recording system. The numerous stimulus types such as the click sound and tone burst are used depending on the user's purpose. Especially, the ALR have been intensively applied not only in auditory studies but also in physiology studies of other neuropsychiatric disorders, which used the customized auditory stimulus paradigms such as the prepulse inhibition, paired click paradigm, or odd ball paradigm.

1.3. Thesis Objectives

There are several limitations in current AEP recording systems, which led to the main objectives of this thesis. Regarding to the commercial EEG and AEP recording devices for research purpose, two or more research instruments should be used and synchronized together because the stimulus generation unit is not integrated (19, 20). In this case, the confirmation of the accuracy of the sound output intensity should be done to use the external instrument. Moreover, obviously it requires separate software control for parameter setting, which could result in inconvenience to users and decrease the system stability. On the other hand, clinical ABR testing devices integrated with the sound generation unit limited the stimulus customization and raw data processing (21, 22). Though some commercial AEP recording platforms for both clinical and research purposes provide considerable flexibility, but they usually require additional modules including the power regulation unit. Moreover, it is noteworthy that most of commercial AEP recording devices are relatively expensive (23). Some research groups have proposed flexible and inexpensive AEP recording platforms to overcome the limitation of commercial devices (21-23). However, their systems still consisted of several separate components such as an analog amplifier, an AD/DA sound card, and an audio amplifier. They also adopted battery-powered circuit to prevent the artifact derived from the power noise, which could lead to the cost and inconvenience of battery replacement.

Thus, the first objective of this thesis was to design a single, flexible and high-fidelity AEP recording system using the adaptor power supply (Chapter 2). For this objective, based on the specification derived from the requirements of the AEP recording system, this thesis proposed a hardware platform including the low-noise AEP recording circuit as well as the accurately calibrated auditory stimulus generation circuit. The power regulation circuit was also integrated to use the adaptor power supply. The software for flexible stimulus and recording parameters control including the real-time signal averaging was also proposed.

The second objective of this thesis was to apply the proposed system to the real clinical research on neurodevelopmental disorders. In this thesis, the ALR recording with a customized stimulus paradigm was performed to investigate the objective evidence of tinnitus. For this, a normative study for the ALR recording using the gap-prepulse inhibition (GPI) paradigm was performed with heathy normal hearing subjects (Chapter 3). Next, a comparative study with tinnitus patients was conducted using the findings of the normative study (Chapter 4).

Chapter 2. AEP Recording System Design

2.1. System Overview

The AEP recording system was designed as a single platform without additional devices such as a data acquisition module and an audio amplifier to increase the system stability and controllability. Figure 2.1 displays a block diagram of the developed system. The system was composed of two main blocks: an AEP recording circuit with analog to digital convertor (ADC) and an auditory stimulus generation circuit with a digital to analog convertor (DAC). The system specification was derived from the AEP characteristics and the American national standard of the specification for audiometers (ANSI S3.6-2004). Detailed specifications for each block are described in following sections. The software was designed to allow users select the type, intensity, and duration of the auditory stimulus and also make a customized structure of the stimulus including the inter-stimuli interval (ISI). The user-designed information of auditory stimulus is transferred to the microcontroller (MCU) #1 on the platform. The MCU #1 performs the parsing operation of the received data and transfers the stimulus data to the DAC. The transfer can be pseudorandomly repeated depending on the user-designed information. After the analog conversion and amplification stage, the auditory stimulus is presented to a subject through the insert earphone. The MCU #1 also sends the trigger signal to the MCU #2 for the synchronization between the stimulus presentation and AEP recording. Moreover, the information of the recording

mode is sent to the MCU #2 to control the ADC sampling rate and recording window length depending on the target AEP signal. The raw AEP signal recorded from surface electrodes on the subject is digitalized in the ADC after the amplification and filter stage, and then transferred to the software via the MCU #2. Using the transferred raw AEP data, the software performs the signal averaging as well as the graph updating and data logging in real-time.

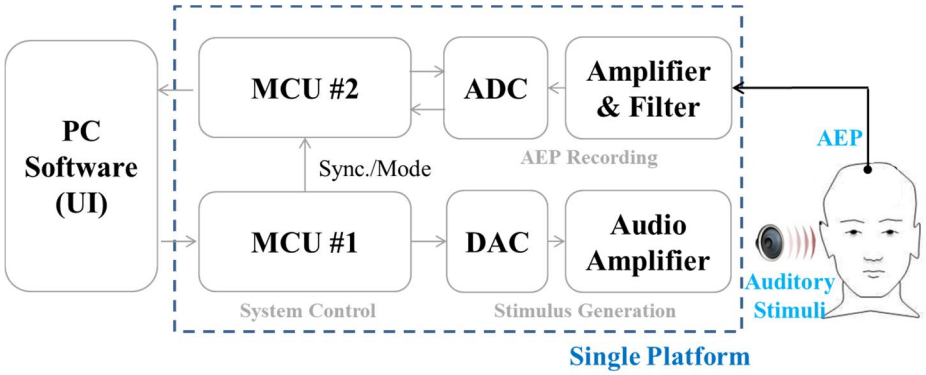

Figure 2.1 Block diagram of the AEP recording system.

2.2. AEP Recording Circuit Design

In this thesis, the system noise level system was targeted to be lower than 0.6 μV_{RMS} in the ABR and ALR recording modes, which is comparable to a clinically-used ABR testing device. Low-noise circuit design techniques to record the high quality of the AEP signal are described in this section.

2.2.1. Analog Front-End and ADC

The raw AEP signal recorded from the electrode should be amplified and filtered in the analog front-end (AFE) circuit due to very low amplitude of the AEP. The development of the low-noise AFE circuit with the appropriate amplification and filtering strategy is the first step to record the high quality of the AEP signal. Figure 2.2 shows the structure of the design AFE circuit. The high input impedance (1 GΩ) operational amplifier was employed as the buffer in the first stage of the AFE circuit. The AEP signal with very low amplitude and wide frequency bandwidth is easily contaminated by the mismatch of skin-electrode contact impedances. High input impedance of the buffer can compensate the mismatch by providing an acceptable common mode rejection. Second, the 1.1 nV/√Hz and 80 nV_{p-p} low-noise operational amplifies and 110 dB high common mode rejection ratio (CMRR) differential amplifiers were employed for the amplification and filtering stages. In the design of the filtering stage, the second-order Butterworth filter using the Sallen-Key topology is implemented following the design recommendation of the AEP recording system (1). The roll-offs of low-pass and high-pass filters

were below 24 dB/octave and 12 dB/octave, respectively. Before the first amplification, the offset voltage from each electrode was removed by the high-pass filter with 1 Hz cut-off frequency to prevent saturation. Moreover, the common mode voltage between the active and reference electrodes, which is derived from ambient electrical field was negatively feed back to the ground electrode on the subject. After first amplification, selectable high-pass (1 or 300 Hz) and low-pass (100 or 3000 Hz) filters were implemented to reflect different frequency bandwidths of the ALR and ABR signal. The full use of the ADC voltage scale and the ADC quantization resolution are very important factors to increase the quality of the recorded signal. Thus, to fully utilize the voltage scale, total amplifying gains were selected to the 12,000 V/V for the ALR signal and 120,000 V/V for the ABR signal under considerations of the raw signal amplitude ranges of the ALR (±200 μV) and ABR (±20 μV) in real recording and the ADC voltage range (±2.5 V). The quantization resolution of the selected ADC was 24 bit, which could achieve the 0.298 μV as an ideal voltage resolution. The effective numbers of bits (ENOB) of the selected ADC were 18.9 and 16.2 bits for selectable 1 kHz and 16 kHz sampling rates, respectively. Thus, when referring to 76 μV noise level (which is the worst case) and ±2.4 V input signal, the estimated signal-to-noise ratio (SNR) was approximately 90 dB, which is enough to record the AEP signal.

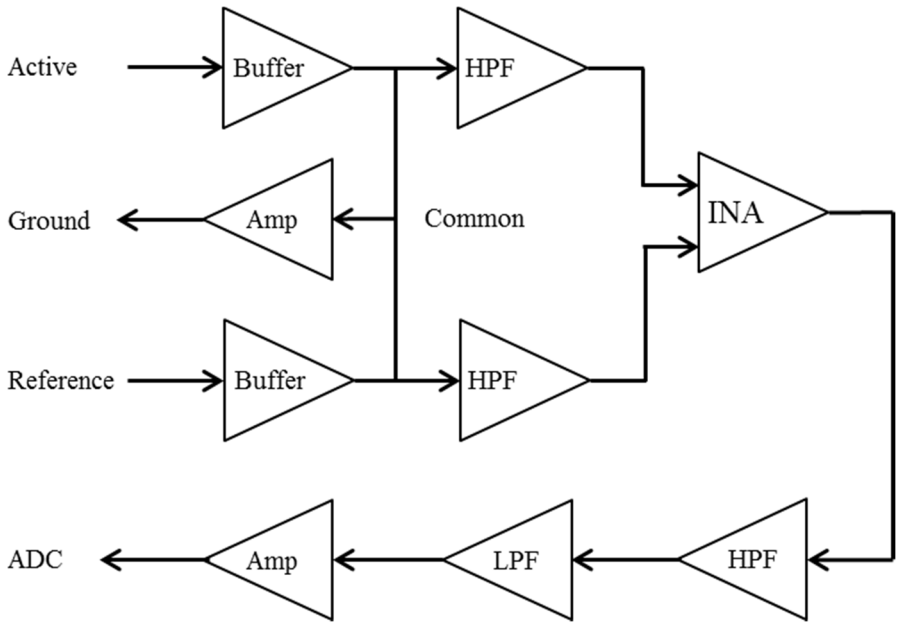

Figure 2.2 Schematic of the analog front-end circuit.

2.2.2. Power Regulation Circuit

Precise power supply to the analog amplifier and ADC reference voltage is also essential to increase the signal quality. Especially for the use of power adaptor, careful regulation scheme is required because the continuous ripple noise generated from the switching regulator in the adaptor power can induce the fluctuation of the input voltage for the amplifier power and ADC reference, which can contaminate the quality of the AEP signal. Thus, switching characteristic should be considered to reduce the effect of the continuous ripple noise. The continuous 52 kHz ripple noise with the 60 mV_{P-P} amplitude was observed on the output of the first switching regulator. Thus, a passive

LC filter with the acceptable size was employed. Then, the multi-stage structure of the linear regulators showing the relatively good characteristic of power-supply rejection ratio (PSRR) at 52 kHz was adopted. The PSRR is defined as the below equation (2.1).

$$PSRR = 20 \times \log\left(\frac{V_{IN\_ripple}}{V_{OUT\_ripple}}\right) \quad (2.1)$$

The estimated noise level of the ±2.5 V output, which is the input for the analog amplifier and ADC reference, was 0.39 nV, which is acceptable for the AEP recording. Figure 2.3 displays the regulation sequence for the reduction of the continuous ripple noise.

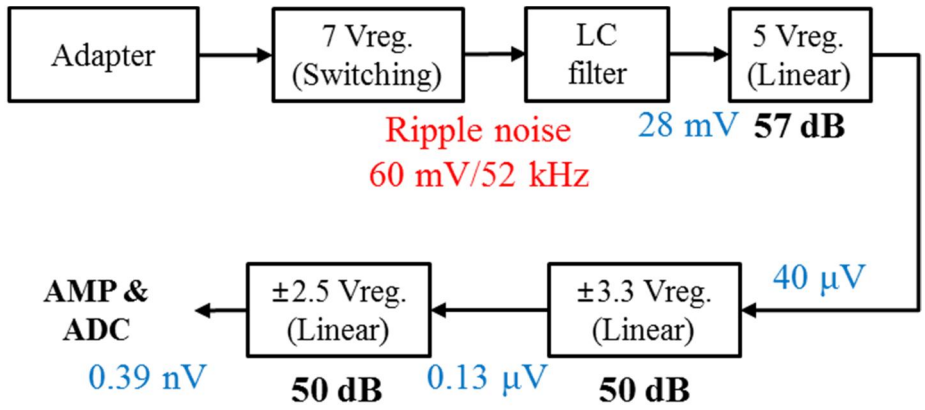

Figure 2.3 Block diagram of the continuous noise reduction sequence.

In the regulation circuit design, the transient response characteristic of the regulators was also considered to integrate the auditory stimulus generation circuit in the AEP recording system. The abrupt current change occurs when presenting the auditory stimulus through an insert earphone. The amplitude of

the current change depends on the intensity of auditory stimulus. The current change can induce the voltage change of the amplifier power and ADC reference based on load and line regulation characteristics. Thus, the estimation of the abrupt voltage change should be performed. In the AEP system, the selected insert earphone for flat high frequency response has the 10 Ω as nominal impedance. Thus, because the maximum driving voltage is the 5 V, the estimated maximum current change is the 500 mA. This current change induced the abrupt noise in the output voltage of switching regulator by the load regulation characteristic. The load regulation is the capability to maintain a constant output voltage when the load varies. The load regulation is defined as the below equation (2.2).

$$\%\ Load\ Regulation = 100\ \times\ \frac{V_{\min\_load} - V_{\max\_load}}{V_{nom\_load}} \tag{2.2}$$

This abrupt change of input voltage in the line regulator can be reduced by the line regulation characteristic. The line regulation is the capability to maintain a constant output voltage when the input voltage varies. The line regulation is defined as the below equation (2.3).

$$\%\ Line\ Regulation = 100\ \times\ \frac{\Delta V_{\mathrm{OUT}}}{\Delta V_{IN}} \tag{2.3}$$

Figure 2.4 displays the sequence of rejection process of the peak noise. The estimated amplitude on the input for the analog amplifier and ADC reference was 28 nV.

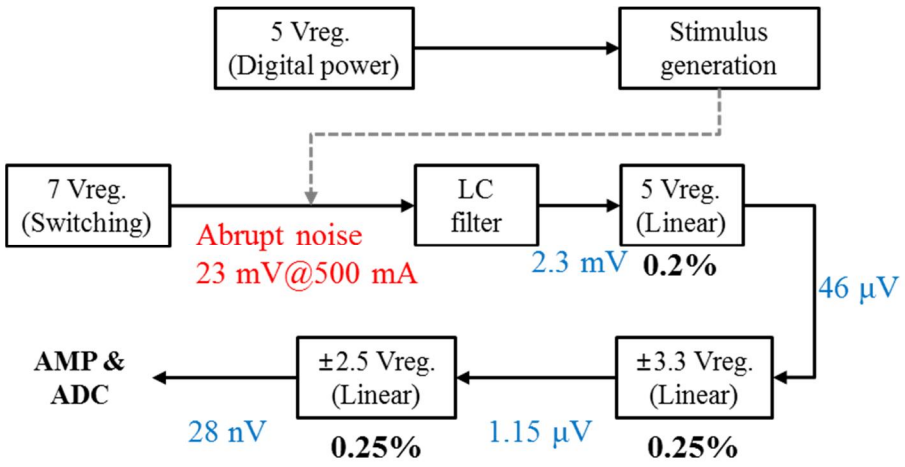

Figure 2.4 Block diagram of the abrupt noise reduction sequence.

2.2.3. Design Considerations for Interference

Further careful grounding for mixed signals was conducted to minimize the interference because the low AEP signal could be vulnerable to noises in ground. A dedicated layer was used for the ground plane to provide large ground area. The analog and digital ground planes as well as the parts were not overlapped. Moreover, the analog ground plane and digital ground plane were connected to refer to a common potential for all voltages through only one point at a mixed-signal part, which is the ADC, called the system star ground. Figure 2.5 displays the layout of the AEP recording system. In this scheme, noisy currents in the digital plane are isolated from the analog plane of the circuit. Moreover, the electrical isolation by the Optocoupler (the yellow area in Figure 2.5) was also employed to minimize the interference from the external device installed the software.

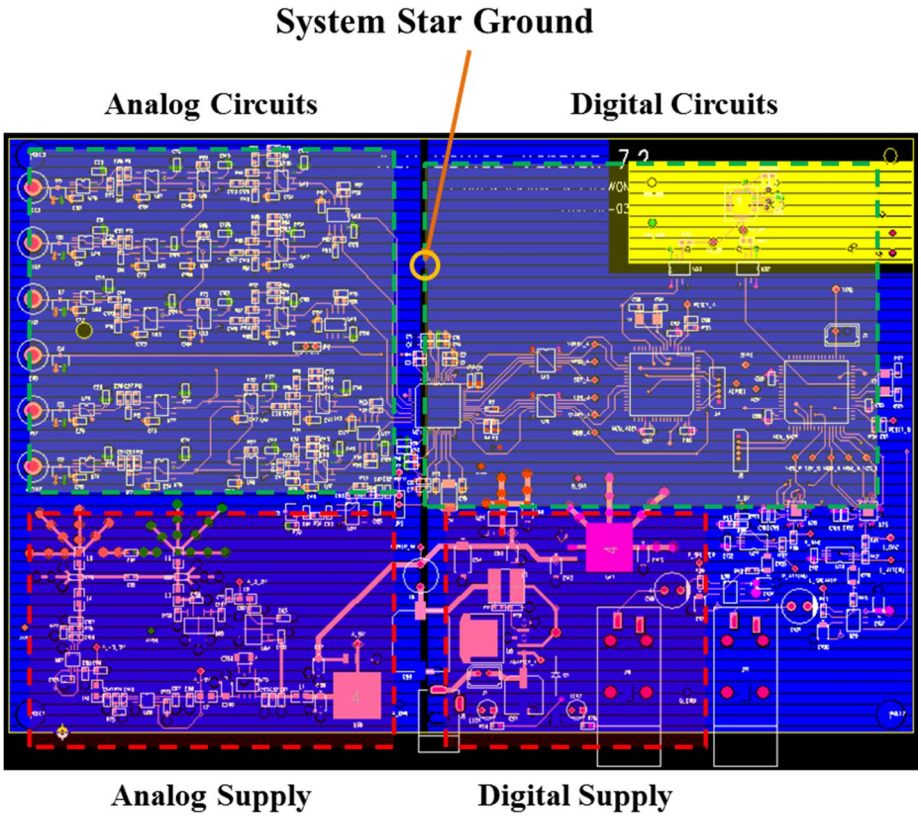

Figure 2.5 Layout of the AEP recording system.

2.3. Stimulus Generation Circuit Design

2.3.1. DAC and Audio Amplifier

Theoretical dynamic range performance of the DAC is limited by resolution. With considerations of the full scale input and quantization error level, the dynamic range determines as the below equation (2.4).

$$Dynamic\ Range = 6.02\mathrm{N} + 1.76\ \mathrm{dB} \tag{2.4}$$

The N is the number of bits of resolution. In many applications, the actual bandwidth of interest is less than the Nyquist bandwidth. If digital filtering is used to filter out noise components outside the bandwidth, then a correction factor, so called process gain can increase the SNR further.

$$Dynamic\ Range = 6.02\mathrm{N} + 1.76\ \mathrm{dB} + 10log_{10}\frac{Fs}{2*bandwidth} \tag{2.5}$$

Because clinical AEP recording systems are usually applied to subjects with hearing loss, it is required relatively wide dynamic range of the output intensity averagely over 100 dB SPL though it can be varied with frequency. Thus, in order to achieve the 100 dB SPL dynamic range as well as the accurate output intensity, the selected DAC featured 18 bits monotonicity with good linearity performance ($\pm$2 LSB max error). Thus, the analog output voltage is expressed as the equation (2.6).

$$V_{OUT} = \frac{V_{\mathrm{REFH}} - V_{\mathrm{REFL}}}{2^{18}} \times b\mathrm{inary\ code} \times \mathrm{buffer\ gain} + V_{\mathrm{REFL}} \tag{2.6}$$

The binary code with the range from 0 to 262143 is controlled by the intensity of stimuli output in the software and the adjustable buffer gain can be set to 1 or 2. The V_{REFH} and V_{REFL} are referred to reference voltages to the DAC chip.

Many auditory disorders are related with the high frequency hearing loss. For example, it has been known that most common tinnitus frequencies in the tinnitus matching test are usually 8 kHz or higher (24). Thus, the sampling frequency of the DAC was selected to 40 kHz to generate the sound with the high frequency such as 12 kHz. Moreover the ER-2 insert earphone was chosen because it has relatively flat high frequency response. For the voltage drive of the ER-2 insert earphone, the dB SPL estimation should be considered in the stimulus generation circuit design. The required output power can be estimated using the V_{OUT} and nominal impedance as the equation (2.7).

$$Output\ Power = \frac{\left(\frac{V_{OUT}}{\sqrt{2}}\right)^2}{nomimal\ impedance} \quad (2.7)$$

The estimated dB SPL can be calculated using above output power and the sensitivity. The sensitivity of the ER-2 insert earphone is the 100 dB SPL for 1.0 V (100 mW with 10 Ω). Thus, estimated dB SPL is expressed as the below equation (2.8).

$$Estimated\ dB\ SPL = 100\ dB\ SPL + 10\ \times \log\left(\frac{Output\ Power}{100\ mW}\right) \quad (2.8)$$

Based on the estimated dB SPL output, the audio power amplifier is selected to deliver 250 mW average power continuously to an 8 Ω output with 0.1 % THD+N using a 5 V power supply. As shown in Figure 2.6, separate DAC and audio amplifiers were used to prevent the interference between left and right channel.

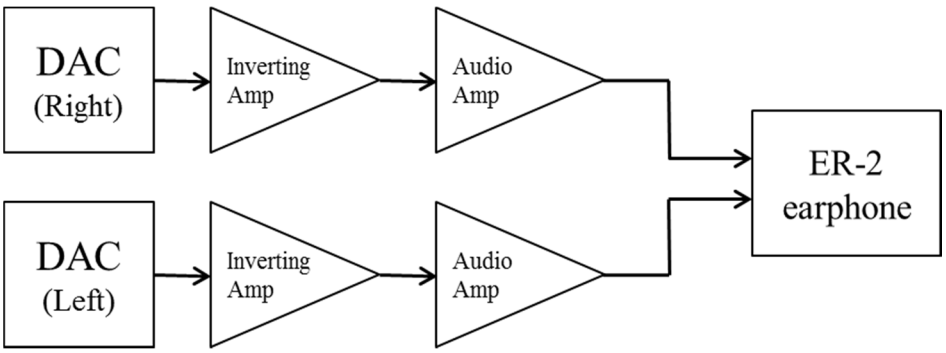

Figure 2.6 Schematic of the stimulus generation circuit.

2.3.2. Stimulus Calibration

The calibration in dB SPL was performed complying with the ANSI S3.6-2004, the specification for audiometers (9.3 Air conduction, insert earphones). All sound output at seven different frequencies (0.2, 0.5, 1, 2, 4, 6.3, and 8 kHz) was calibrated by a sound level meter (Type 2250 Sound Level Meter, Bruel & Kjaer, Nærum, Denmark) with an ear simulator (IEC Ear Simulator RA0045, G.R.A.S. Sound & Vibration, Holte, Denmark) in a soundproof booth as shown in Figure 2.7. The RA0045 complies with the international

requirements of IEC 60318-4 (occluded-ear simulator for the measurement of earphones coupled to the ear by ear inserts), which shows very similar response with the Zwislocki coupler. The dB SPL to equivalent dB HL conversion should be conducted to reflect hearing ability in dB HL. This procedure was based on the conversion table of the insert earphone in the Zwislocki coupler. The calibration results for all frequencies were implemented by the form of a lookup table in the software.

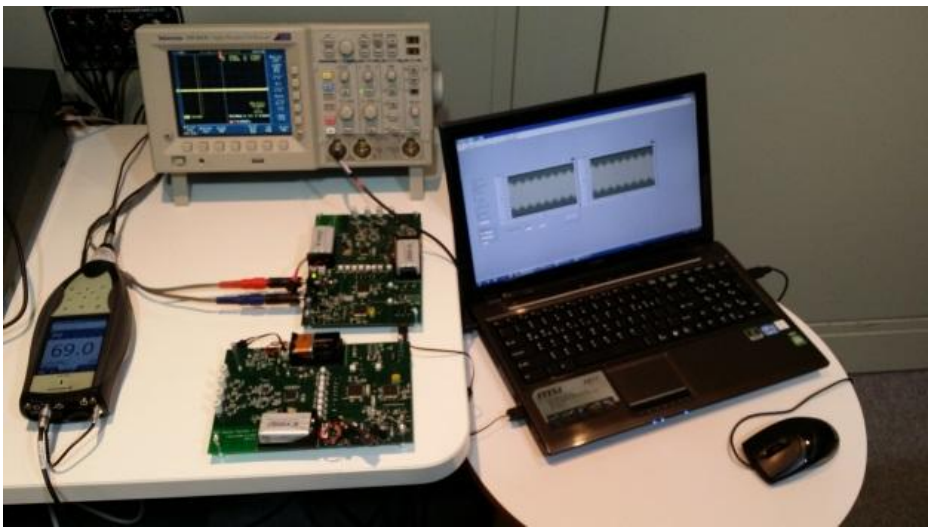

Figure 2.7 Picture of the calibration setup.

2.4. Software Design

2.4.1. User Interface

Figure 2.8 is a screenshot of the developed user interface (UI) for the auditory stimulus setting and the AEP signal processing, which was implemented in the PC software by Labview (National Instruments, Austin, TX). Detailed specifications of each block are described in following sections. Users can select various stimulus parameters such as the output direction (left or right), type, intensity, and stimulus duration. Moreover, if users provide the information of the subject's hearing level, the PC software calculates the appropriate dB SL by the implemented lookup table. The stimulus customization such as the prepluse inhibition paradigm is also available. For the AEP recording, users can select the number of averaging and the threshold amplitude for the real-time noise rejection. The averaged waveform as well as the raw waveform is updated in real-time.

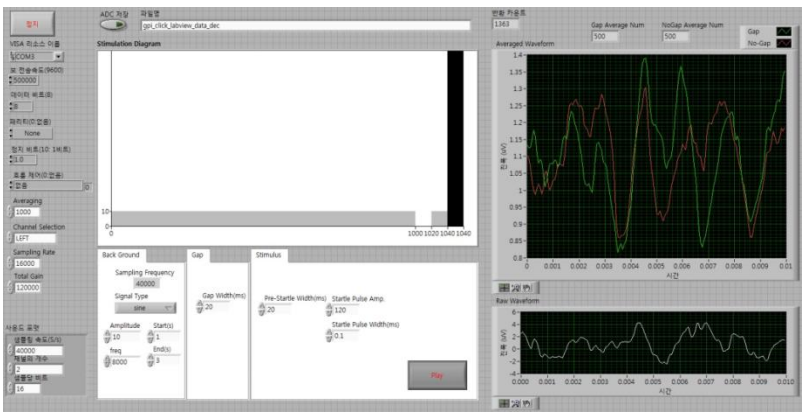

Figure 2.8 Screenshot of the user interface.

2.4.2. Real-Time Data Processing Structure

Real-time AEP data processing can provide many benefits in clinic. First, the required test time can be decreased because an operator can decide the required number of stimulus repetitions by monitoring the trend of the recorded AEP signal. The required repetitions can vary depending on the subject's intrinsic characteristic or ambient noise situation. Moreover, the real-time monitoring can reduce the chance of the mistrial contaminated by abrupt noises such as the motion artifact or electrode-off. Finally, the real-time processing is also beneficial for the cases to find the stimulus condition in test by test. Thus, the parallel multi-loop processing was adopted to achieve the real-time data processing. As shown in Figure 2.9, the developed multi-loop processing is based on the producer and consumer design pattern. If the data receipt (produce) and the data process (consume) are included in the same loop, the next receipt of data cannot be performed before finishing the data process. Thus, loop separation approach by the enqueue and dequeue operations was required. This design enables the data sharing between multiple loops which run with different running rates. The data transfer between the producer and consumer loops is done by additional data queues. These queues also provide buffering for different rates between loops. In Figure 2.9, first queuing up is done in the data acquisition loop. The second loop dequeues the data from this queue and performs the parsing and integer conversion, and put the result into the second queue. The third loop dequeues the data from the second queue and then performs the data accumulation up to

the predefined length, signal averaging, and graph updating. In the third loop, the accumulated data is transferred to the third queue, which is consumed for the data logging in the final loop. This multi-loop design and the queue communication technique allowed the real-time AEP processing.

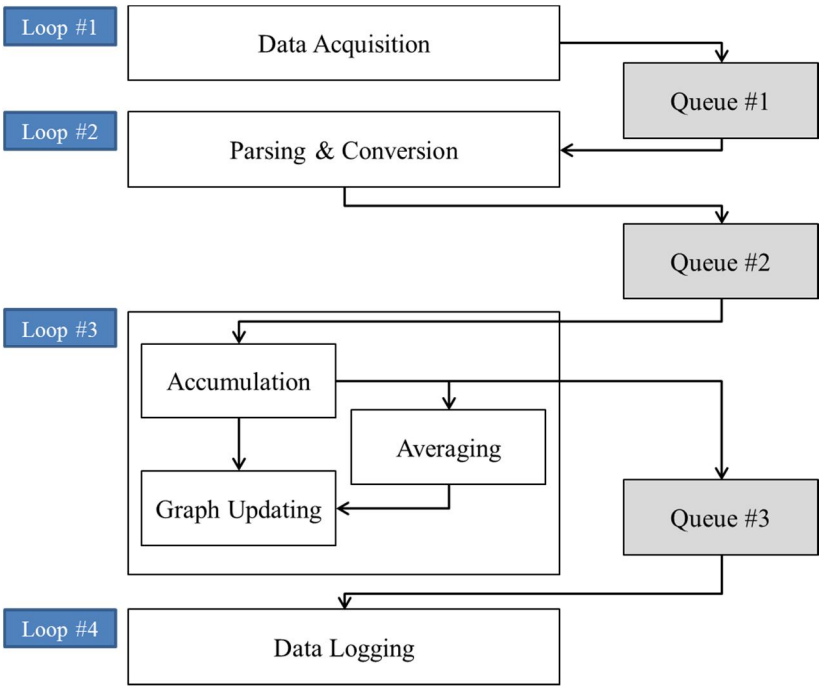

Figure 2.9 Block diagram of the parallel loop processing.

2.5. Experimental Setup

The system noise level was evaluated with the electrically-short of active, reference, and ground electrodes. Six repetitive tests were performed for two different system boards. For each test, the raw data noise level was assessed to compare to the commercial single platform ABR testing device. For the ABR recording mode, the concatenated raw data length was 5 sec (10 ms x 500 repetitions). For the ALR recording mode, the concatenated raw data length was 80 sec (800 ms x 100 repetitions). The averaged data noise level was also assessed for the comparison with the ABR and ALR amplitudes.

The stimulus output accuracy was evaluated using pure tone signals with various frequencies (200, 600, 1000, 2000, 3000, 4000, 6000, 8000, and 12000 Hz). In the ANSI S3.6, the error of the output intensity should be below 3 dB SPL in the 0.5 to 4 kHz range and 5 dB SPL in other frequencies. The frequency tolerance should meet ±1 % up to 8 kHz and ±2 % over 8 kHz range. The maximum output level was also assessed referring to 100 dB SPL as the specification.

Finally, real ABR and ALR signals were recorded from 3 normal hearing subjects. Test and retest sessions were performed for each subject. As shown in Figure 2.10, subjects were seated in a soundproof booth and were requested to refrain from moving too much. The skin preparation was conducted to lower the skin-electrode impedance below 5 kΩ. A 10 mm gold disc electrode (F-E5GH, Grass Technologies, Warwick, RI) with conductive

paste was placed on the vertex (Cz) for the active electrode (25). Adhesive silver-silver chloride electrodes (Kendall^{TM} 100 series, Covidien llc, Mansfield, MA) were placed on the ipsilateral mastoid (A1 or A2) and the forehead (Fpz) as a reference electrode and a ground electrode, respectively. In the ABR recording, alternative click sound (condensation and rare faction) with 0.1 ms duration was presented in 90 dB SPL. The stimulus rate was 11.1 Hz. In the ALR recording, 1 kHz tone burst with 20 ms duration was presented in 65 dB SL. The intervals between tone burst stimuli were pseudo-randomly varied between 1 and 3 s.

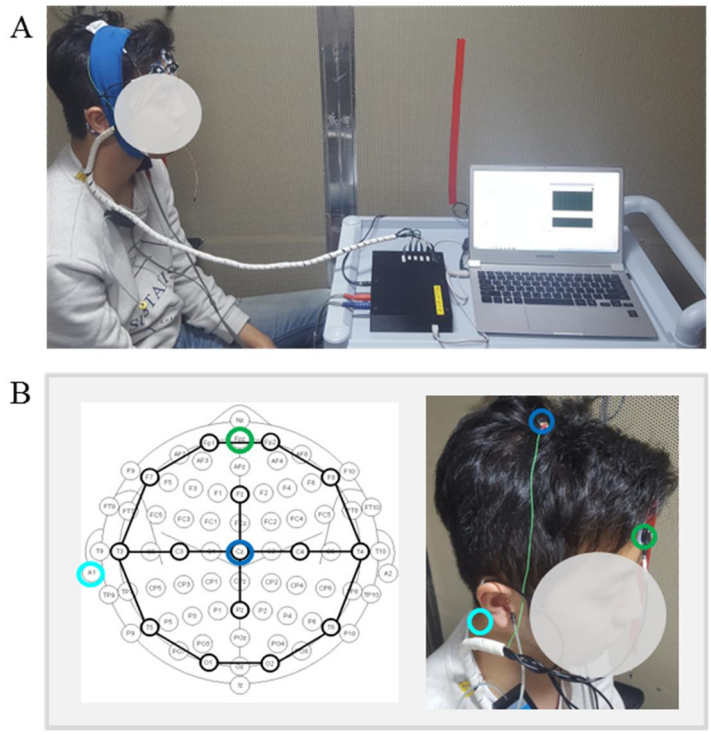

Figure 2.10 Pictures of (A) experimental setup for the AEP recording and (B) electrodes location.

2.6. Results and Discussion

2.6.1. System Noise Level Evaluation

Figure 2.11 displays the developed single AEP recording platform (202 mm x 133mm). The required external components are only electrodes, an insert earphone, a power supply adaptor, an USB data cable, and a laptop installed the developed software.

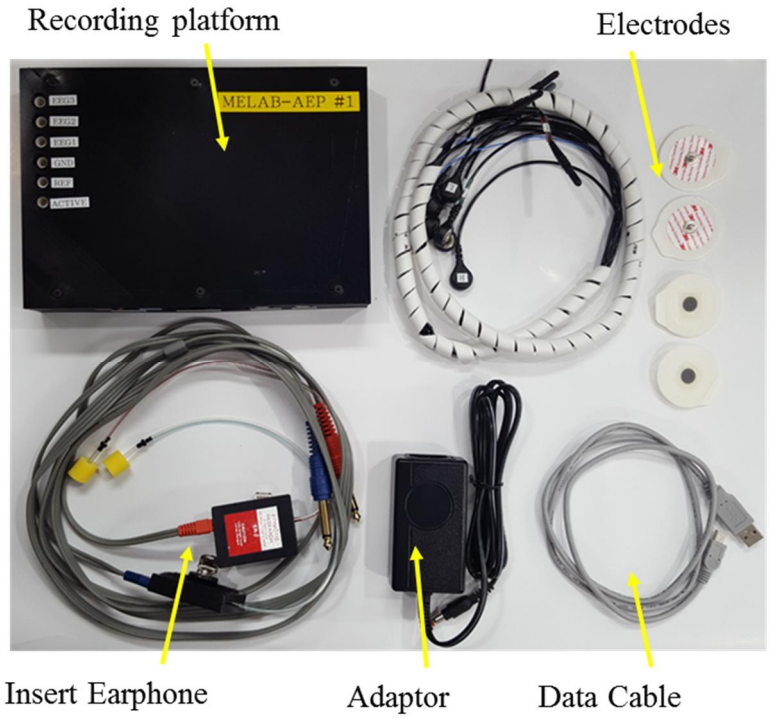

Figure 2.11 Picture of hardware components of the AEP recording system.

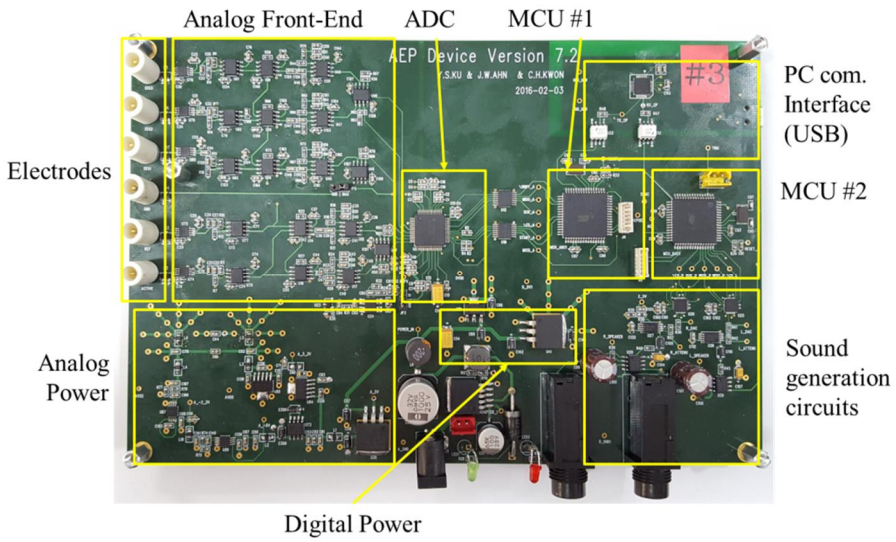

Figure 2.12 Picture of the circuit board of the AEP recording system.

Figure 2.12 shows the circuit board of the AEP recording system. The analog and digital parts are separately placed, centered at the ADC. The evaluation result of the system noise level is given in Table 2.1. In the 6 repetitive tests, the mean noise levels of raw signals were 0.567 and 0.592 μV_{RMS} for the ABR and ALR recording modes, respectively, which were comparable to 0.6 μV_{RMS}, the noise level of clinically-used ABR testing device. This ABR specialized testing device is composed of a single recording platform. The system noise level can be reduced by using the battery or external power regulation unit. Moreover, the amplifier with lower input noise can be used as the buffer. However, in general, there is a performance trade-off between the input impedance and input noise of the amplifier. Low input impedance could be susceptible to mismatching of the skin-electrode impedances, which can seriously degrade the CMRR of the AEP recording system. Thus, the

appropriate design strategy of the trade-off between the high input impedance and low input noise should be required.

Table 2.1 System noise level evaluation

	ABR Mode	ALR Mode
Raw Noise Level (SD) (μV_{RMS})	**0.567** (0.011)	**0.592** (0.03)
Averaged Noise Level (SD) ($\mu V_{RMS}/\mu V_{P-P}$)	**0.026** (0.002) / **0.113** (0.013)	**0.072** (0.014) / **0.344** (0.05)

Figure 2.13 displays the noise levels of averaged signals. For the ABR recording mode with 500 repetitions, the mean noise level of the averaged signal was 0.113 μV_{P-P}. For the ALR recording with 100 repetitions, the mean noise level of the averaged signal was 0.34 μV_{P-P}. When comparing to the general amplitudes of the ABR (0.5 μV_{P-P}) and ALR (1~10 μV_{P-P}), the averaged noise amplitudes were considered to be acceptable for the AEP recording.

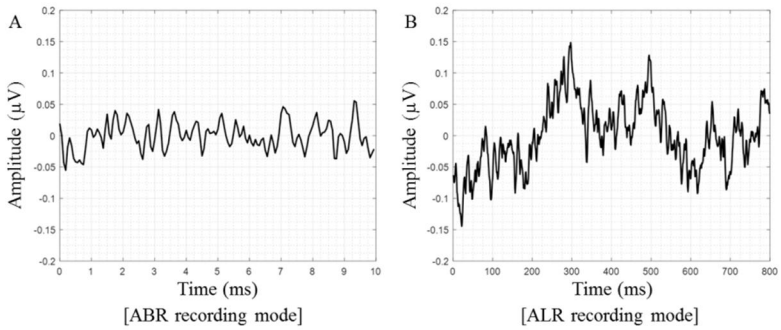

Figure 2.13 Averaged noise waveforms in the (A) ABR and (B) ALR recording modes

2.6.2. Stimulus Accuracy Evaluation

Figure 2.14 displays various types of the auditory stimuli presented from the AEP recording device. The pure tone sounds with various frequencies, white noise, click sound, and tone burst were confirmed with their exact durations and intensities on an oscilloscope. Moreover, it was confirmed that a customized pattern using the pure tone and white noise was successfully presented. The evaluation results of the sound generation are given in Table 2.2. Regarding to the accuracy of intensity, the errors for all test frequencies was below ±1.5 dB SPL, which met the requirements of the ANSI S3.6 (below 3 dB SPL in the 0.5 to 4 kHz range and 5 dB SPL in other frequencies). The frequency accuracies were below ±1% at all test frequencies, which also met the ANSI S3.6 (±1 % up to 8 kHz and ±2 % over 8 kHz range). The maximum intensities of all frequencies were higher than the targeted 100 dB SPL.

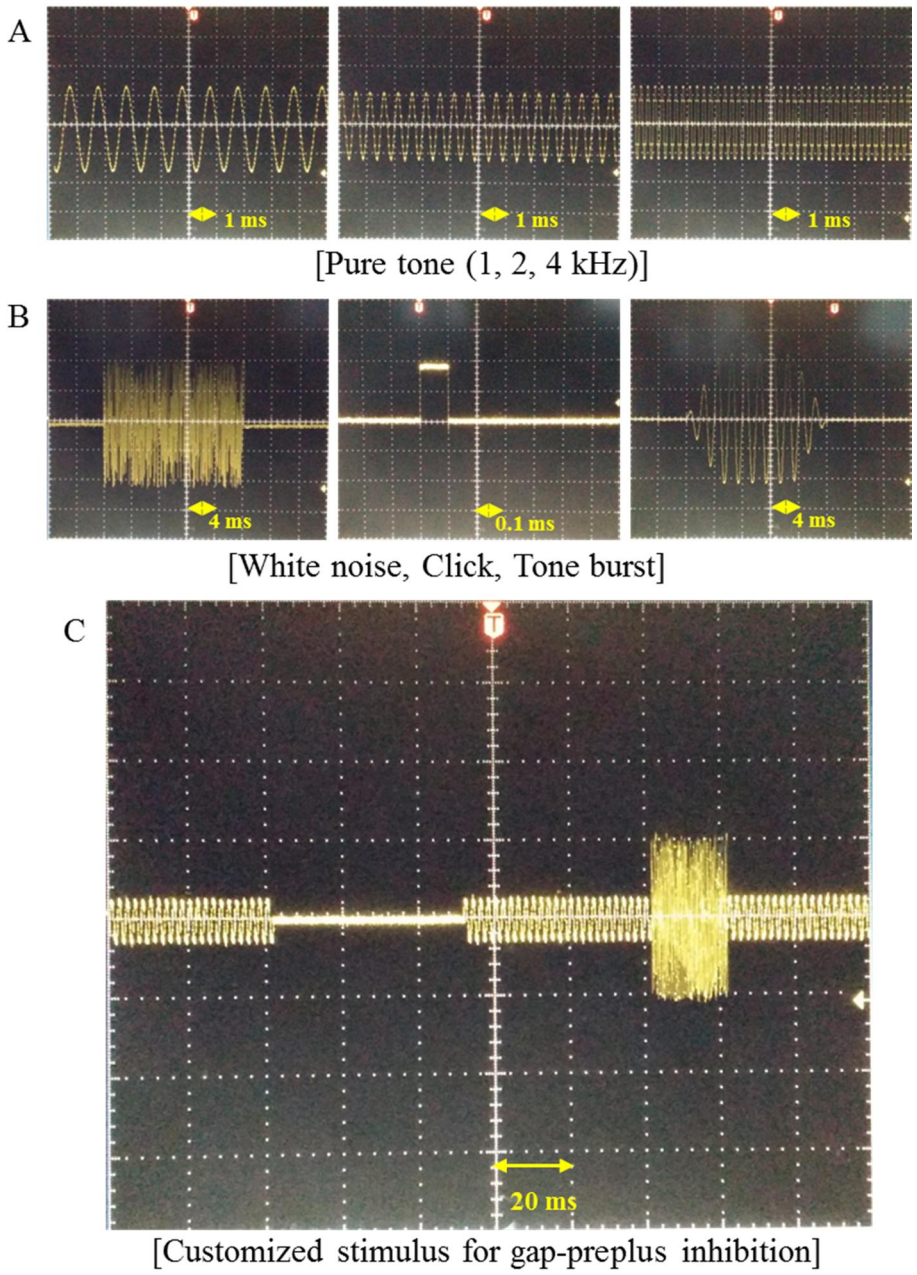

Figure 2.14 Waveforms of the stimulus output (A) continuous pure tones (1, 2, and 4 kHz), (B) 20ms white noise, 0.1 ms click sound, and 20 ms tone burst, and (C) customized stimulus for gap-prepulse paradigm

Table 2.2 Auditory stimulus evaluation

Freq. (Hz)	Intensity Accuracy (dB SPL)			Maximum intensity (dB SPL)	Frequency Accuracy (Hz)	
	Target Value	Measured Value	Error	Measured Value	Measured Value	Error
200	70	70.4	0.4	101.9	199.2	-0.8 (-0.4%)
	80	80.5	0.5			
	90	90.9	0.9			
	100	100.2	0.2			
600	70	70.8	0.8	101.4	595.4	-4.6 (-0.7%)
	80	81.0	1.0			
	90	90.3	0.3			
	100	100.7	0.7			
1000	70	70.6	0.6	103.3	996.9	-3.1 (-0.3%)
	80	80.9	0.9			
	90	90.2	0.2			
	100	100.6	0.6			
2000	70	70.7	0.7	105.3	1990.0	-10.0 (-0.5%)
	80	80.9	0.9			
	90	90.1	0.1			
	100	100.5	0.5			
3000	70	70.8	0.8	105.8	2985.8	-14.2 (-0.5%)
	80	79.0	-1.0			
	90	89.0	-1.0			
	100	99.1	-0.9			
4000	70	69.9	-0.1	109.7	3975.2	-24.8 (-0.6%)
	80	79.8	-0.2			
	90	89.9	-0.1			
	100	100.0	0			
6000	70	70.1	0.1	109.2	5973.7	-26.3 (-0.4%)
	80	80.0	0			
	90	90.1	0.1			
	100	100.5	0.5			
8000	70	69.6	-0.4	102.0	7966.2	-33.8 (-0.4%)
	80	79.5	-0.5			
	90	89.8	-0.2			
	100	100.1	0.1			
12000	70	69.8	-0.2	107.0	12025.0	25.0 (0.2%)
	80	78.7	-1.3			
	90	88.9	-1.1			
	100	99.0	-1.0			

2.6.3. Real AEP Recording

Figure 2.15 displays real ABR and ALR waveforms recorded from 3 normal hearing subjects. For the ABR waveforms, clear ABR morphologies with around 0.5 μV_{P-P} amplitude were shown in all subjects. Typical peaks (I, III, and V) of the ABR were confirmed and were reproducible at retest in all subjects. Similarly for the ALR waveforms, clear ALR morphologies with around 10 μV_{P-P} amplitude were also shown in all subjects. Typical peaks (P1, N1, P2, and N2) of the ALR were confirmed and were reproducible at retest in all subjects.

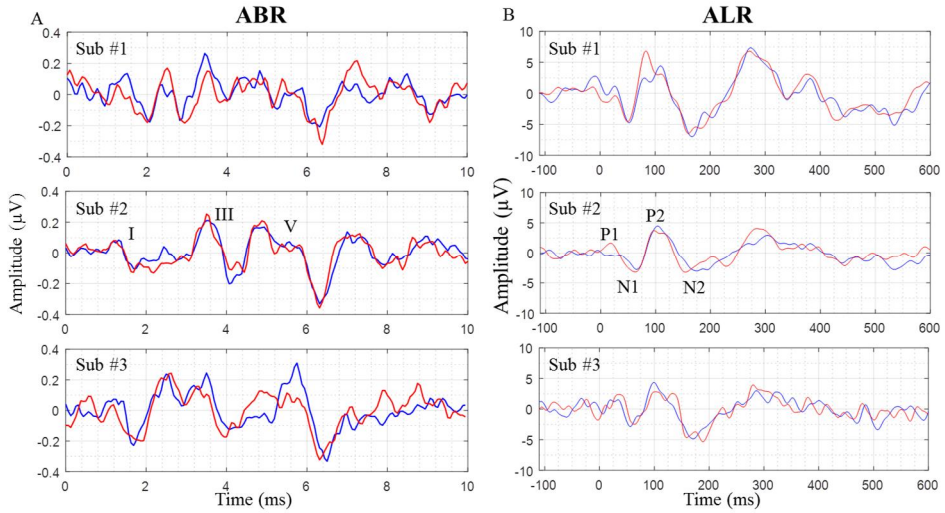

Figure 2.15 Waveforms of (A) the recorded ABR and (B) ALR.

2.7. Conclusion

A high-fidelity AEP recording system was developed in terms of the low system noise level and precise stimulus generation. The system noise level was comparable to a clinically-used commercial ABR testing device. The accuracies of the stimulus intensity and frequency met the requirements of the international standard for the sound presentation. Moreover, the real ABR and ALR recording was confirmed with good reproducibility. The proposed AEP device could also provide flexible stimulus parameter setting and real-time AEP data processing, which are useful for various clinical and research applications of the AEP.

* Portions of this chapter were published in Proc.36th Int. IEEE EMBS Conference (Ku Y, Ahn JW, Kwon C, Suh M-W, Lee JH, Oh SH, et al. A programmable acoustic stimuli and auditory evoked potential measurement system for objective tinnitus diagnosis research. Conference Proceedings of the IEEE Engineering in Medicine and Biology Society;2014:2749-52.) (26)

Chapter 3. Clinical Application:

A Normative Study

3.1. Introduction

Tinnitus is the brain's perception of sound with no physical acoustic stimulus in the ears. The prevalence of tinnitus has been to be estimated between 10% and 15% (27, 28). Currently, the clinical diagnosis of tinnitus relies on subjective methods, such as a tinnitus evaluation questionnaire or tinnitogram; attempts have been made to develop a more robust assessment of tinnitus (29-31). Since the gap-startle paradigm was first proposed as a tinnitus screening tool in rats, this behavioral method has been intensively adopted and evaluated in animal studies (32-43). In the gap-startle paradigm, gap-prepulse inhibition of the acoustic startle reflex (GPIAS) is used to estimate gap detection ability of animals. If the stimulus with a silent gap prior to a loud startling sound fails to inhibit the acoustic startle reflex, it is assumed that the animal could not detect the gap because tinnitus fills the gap. Although this hypothesis has been argued in animals (44), the gap-startle paradigm was recently evaluated in humans using eye blinking as the startle reflex response (30). In their study, gap inhibition significantly decreased in subjects with mild, high-pitched tinnitus compared to control subjects without tinnitus. However, whether tinnitus filled the gap in humans was still unclear because the startle reflex was also suppressed at low-frequency background noise,

which did not match the tinnitus pitch. Another recent human study investigating the "filling in the gap" concept reported that there was no significant difference in gap detection ability between tinnitus subjects and controls without tinnitus when presenting silent intervals in background narrow band noise (45). They suggested that this discrepancy may be due to the difference in the related neural areas between the startle reflex (subcortical auditory structures) and the psychoacoustic task (auditory and motor cortex).

In this thesis, the feasibility of auditory evoked potential (AEP) was investigated to be used as the response of the gap-prepulse inhibition in humans. The hypothesis was developed based on the results of previous research that reported the relationship between tinnitus and auditory cortical reorganization in response to nerve damage (39, 46-49). As described in Chapter 1, the auditory late response (ALR), occurring after 80 ms to 500 ms, reflects the complex auditory cortical processing involved in the auditory- and frontal-cortex (50-53). Therefore, it was hypothesized that if tinnitus is correlated with neural plasticity changes in the auditory cortex, AEP, especially ALR, would reflect those changes in the response of the gap-prepulse inhibition (GPI). Moreover, in terms of objective tinnitus measurement in humans, involuntary ALR is a good candidate for a response index of the GPI because behavioral or cognitive responses, such as eye blink or gap perception, can be manipulated by a subject's intention.

Pre-pulse inhibition (PPI) is the suppression of the startle reflex in response to a sudden intense stimulus by a weaker pre-stimulus preceding the startling stimulus by 30-500 ms (54). The PPI of acoustic startle responses such as an eye blink has been commonly used as an objective measure of the temporary adaptation to a strong stimulus (i.e., sensorimotor gating) , which serves a protective mechanism against sudden or threatening stimuli stimuli (55, 56). The inhibitions of AMLR and ALR by a weaker pre-pulse, called sensory gating, have also been reported in several previous studies (11, 57-62). The neural mechanism for the modulation of cortical responses was thought to be different from that involved in the startle reflex (63). Recent studies, however, have suggested that there may be some shared mechanisms between the PPI of the startle reflex and the PPI of cortical responses (11). Because the GPIAS that uses a temporal gap as a pre-pulse was found in both animals and humans (30, 64-67), it was similarly hypothesized that the ALR peak amplitudes in response to an intense auditory stimulus following a silent gap in subjects without tinnitus would be inhibited more than amplitudes in response to an intense sound-only stimulus, called a gap-intense sound paradigm. In tinnitus patients, however, the reorganization of the auditory cortex would impair the acknowledgment of a silent gap for an incoming intense auditory stimulus.

. First of all, it was necessary to understand the GPI features of typical ALR peaks in healthy subjects before conducting a comparative study of GPI indices with tinnitus patients. The indices that showed significant inhibitions

in normal subjects can be compared to those of tinnitus patients in future studies. Accordingly, the suppression of typical ALR peaks (baseline-to-peak and peak-to-peak) was examined when presenting the stimuli of the gap-intense sound paradigm with high- and low-frequency background noises. Second, the change in GPI ratios from the beginning to the end of the experiment was analyzed. The intervals between the startle stimuli in the gap-startle paradigm in previous studies were greater than 10 s to prevent stimulus prediction by animals and human subjects (30, 41). Because many stimuli (< 200) are repetitively presented in a general ALR test for signal-to-noise improvement (1), the required test time dramatically increases when performing the gap-intense sound paradigm with ALR measurement. Thus, it was aimed to elucidate the minimum required trial number for a stable GPI response in normal-hearing subjects. Lastly, the effects of stimulus parameters such as gap length, inter-stimulus interval (ISI) length, and background noise amplitude on the GPI ratio were assessed. The variation of GPI indices with stimulus parameters should be investigated to determine the stimulus parameter criteria that can reveal differences between healthy subjects and tinnitus patients in the comparative study.

3.2. Methods

3.2.1. Subjects

Twenty-seven adults (eight females) with a mean age of 26.9 years and an age range of 21 to 37 years participated in this study. All subjects reported that they had never suffered from tinnitus or any other neurological diseases. Pure tone air-conduction threshold tests were performed in a soundproof booth using standard audiometric procedures. In all subjects, normal hearing (below 25 dB HL in the 0.25 to 8 kHz frequency range) was verified by an audiogram before the experiment. The study was approved by the Institutional Review Board of Seoul National University Hospital, and all subjects provided written consent.

3.2.2. General Procedure

ALR was recorded in response to auditory stimuli of the gap-intense sound paradigm with a high-frequency (8 kHz) background noise, which is generally well matched to the tinnitus frequency. After a 5-minute break, ALR to the stimuli containing a low frequency (600 Hz) background noise were measured. The retest session was performed within 1 to 2 weeks, and the order of background noise frequencies was randomized. In the *stimulus parameter analysis*, ALR measurements were performed under 11 different stimulus conditions. All subjects were included in the ALR measurement with the gap-

intense sound paradigm, and eight subjects participated in the *stimulus parameter analysis.*

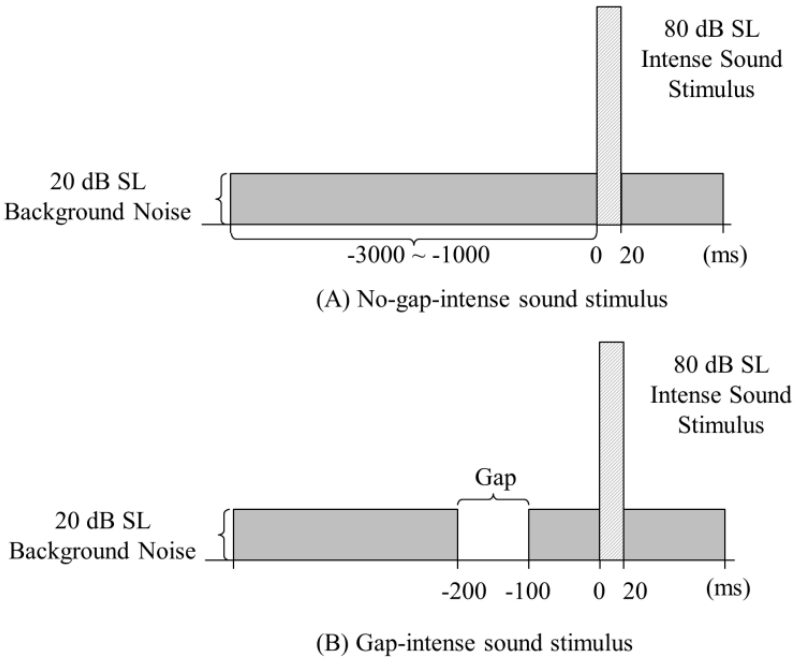

Figure 3.1 Stimulus structure of (A) No-gap-intense auditory stimulus and (B) Gap-intense auditory stimulus. No-gap-intense auditory stimulus contained 80 dB SL intense auditory stimulus (20 ms/1 kHz tone burst) with 20 dB SL continuous background noise (8 kHz or 600 Hz pure tone). Gap-intense auditory stimulus contained the same intense auditory stimulus and background noise, but a 100 ms temporal gap was inserted 100 ms prior to the onset of the intense auditory stimulus. Auditory late response (ALR) was recorded after presenting the intense auditory stimulus. The intervals between trials were pseudo-randomly varied between 1 and 3 s.

3.2.3. Stimuli

As shown in Figure 3.1, the stimulus was composed of a continuous background noise and an intense auditory stimulus that were generated in dB SL scale to reflect the hearing ability of each individual subject. Continuous pure tone background noises were set at 20 dB SL for both high and low frequencies. The intense sound was a 20 ms/1 kHz tone burst with rise and fall times that were 5 cycles in duration. The intensity of the intense sound was 80 dB SL. The duration of inserted gaps in the gap-intense auditory stimulus was 100 ms, which was long enough to evoke the auditory event-related potential in response to temporal gaps (68). The time between a temporal gap and an intense auditory stimulus (i.e., the ISI) was set to 100 ms, which is very close to the optimal duration for sensorimotor and sensory gating (69-71). The intervals between the intense auditory stimuli were pseudo-randomly varied between 1 and 3 s to minimize the temporal prediction of the intense auditory stimulus presentation. The number of sweeps for gap-intense auditory stimuli and no-gap-intense auditory stimuli was 150, and their sequences were pseudo-randomly generated. The sessions did not exceed approximately 10 minutes. In the stimulus parameter analysis, four gap durations (20, 50, 100, and 200 ms) were tested with 100 ms ISI and 20 dB SL/8 kHz background noise. The GPI trend was also monitored for each ISI (20, 50, 100, and 200 ms) with 100 ms gap duration and 20 dB SL/8 kHz background noise. Finally, three different background intensities (5, 20, and 35 dB SL) were tested with 100 ms gap duration and 100 ms ISI. All

stimuli were generated by the developed AEP recording system described in Chapter 2. The ER-2 insert earphone (Etymotic research INC., Elk Grove Village, IL) was used for the undistorted high frequency sound output. All stimuli were calibrated for different frequencies (200, 500, 1000, 2000, 4000, 6300, and 8000 Hz) with a sound level meter (Type 2250 Sound Level Meter, Bruel & Kjaer, Nærum, Denmark) in a soundproof booth.

3.2.4. ALR Recording

All ALR recordings were performed in a soundproof booth. Subjects were seated in a comfortable reclining armchair, and they passively listened to auditory stimuli. They were instructed to ignore the stimuli and watch the silent video clips, which were text-based educational videos on history, science, or medicine. They were also requested to refrain from falling asleep and moving too much. The test started with skin preparation to lower the skin-electrode impedance. The impedances were kept below 5 kΩ. The impedance differences between electrodes were maintained at less than 2 kΩ. A 10 mm gold disc electrode (F-E5GH, Grass Technologies, Warwick, RI) with conductive paste was placed on the vertex (Cz) for the non-inverting electrode according to the ALR measurement guidelines (Hall, 2006). Disposable, adhesive silver-silver chloride electrodes (KendallTM 100 series, Covidien llc, Mansfield, MA) were located on the ipsilateral mastoid (A1 or A2) and the forehead (Fpz), serving as an inverting electrode and a ground electrode, respectively. The pre-stimulus time for the baseline correction was 200 ms

before the onset of the intense auditory stimulus. The 600 ms epoch, which was long enough to include typical ALR peak components (N1, P2, and N2), was analyzed. The analog band-pass filter with a cutoff frequency of 1 to 100 Hz was applied before the analog-digital conversion (ADC), and the sampling rate of ADC was 1000 Hz. In total, 150 epochs for each stimulus condition (gap-intense auditory stimulus and no-gap-intense auditory stimulus) were recorded, and any epochs with amplitudes greater than ± 100 μV were excluded from averaging (less than 10 % in each measurement). Two averaged waveforms were computed relative to the gap-intense auditory stimuli and the no-gap-intense auditory stimuli.

3.2.5. Data Analysis

The ALR analysis included the comparison of the baseline values to the N1, P2, and N2 peak amplitudes (baseline-to-peak amplitudes) between two averaged waveforms relative to the gap-intense auditory stimuli and the no-gap-intense auditory stimuli as shown in Figure 3.2. P1 was excluded from the analysis because of the ALR overlapping components that originated from onsets and offsets of gaps prior to the intense auditory stimulus onsets. Moreover, the two peak-to-peak amplitudes, the N1-P2 complex and the P2N1 complex, were examined. The amplitudes of all peaks were computed using an automated peak detection algorithm in a predefined latency region

for each peak (N1: 60–150 ms, P2: 100–250 ms, N2: 150–300 ms). The epochs that showed abnormal ALR morphologies (no N1 or P2 peak) were excluded from the analysis (less than 15 % in each session). The paired sample *t*-test was applied to determine the differences in peak amplitudes between the gap condition and the no-gap condition. Test-retest reliability was analyzed using Pearson's correlations at each background frequency. GPI was defined as the reduction ratio of the amplitudes (GPI=Gap/No-Gap), in which Gap represents the amplitude of the averaged waveform of the gap-intense auditory stimuli, whereas No-Gap represents the amplitude of the averaged waveform of the no-gap-intense auditory stimuli. Three-way ANOVAs with repeated measures were conducted. The independent variables included sessions ('Test' or 'Retest'), background noise frequencies ('8 kHz' or '600 Hz'), and gap presence ('Gap' or 'No-Gap') as within-subjects factors for all baseline-to-peak amplitudes and peak-to-peak amplitudes. One-tailed one sample *t*-tests were run as post hoc comparisons to determine whether GPI ratios at high- and low-frequency background noises in test-retest sessions were smaller than 1.0, where the value 1.0 indicates no difference between the gap- and no-gap-intense sound responses. Further investigations were performed for peak-to-peak amplitudes that only showed relatively higher test-rest correlations and gap/no-gap differences compared to baseline-to-peak amplitudes. In the *cumulative average trend analysis*, GPI ratios of peak-to-peak amplitudes were calculated from the beginning to the end of a measurement with the increase of the trial number by 10. One-way ANOVAs

with repeated measures were used to examine the changes in peak-to-peak amplitudes, with Bonferroni's correction adopted for pairwise comparisons. To assess the effect of stimulus conditions on GPI ratios, a one-way ANOVA with repeated measures was also performed separately on each condition, and then Bonferroni's correction was adopted for multiple comparisons in the post hoc test.

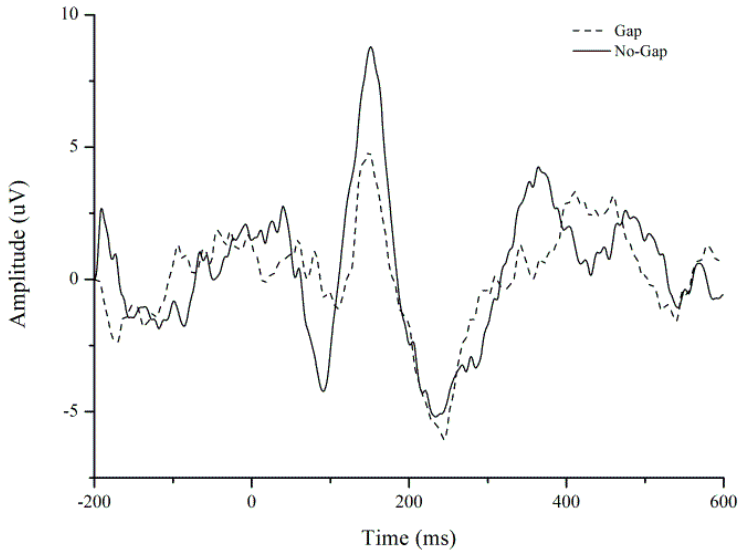

Figure 3.2 A typical ALR waveform in response to gap-intense and no-gap-intense auditory stimuli. A negative peak, N1, was generally found between 90 and 150 ms from the onset of the intense auditory stimulus. A positive peak, P2, was found in the period from 160 to 200 ms, and it was followed by a second negative peak, N2, between 200 and 300 ms. Note the suppressed amplitudes of ALR peaks with gap- intense auditory stimuli.

3.3. Results

3.3.1. N1, P2, N2, N1-P2, and P2-N2 amplitudes

As shown in Figure 3.3, at 8 kHz background noise, the overall gap-intense sound responses showed smaller amplitudes than those of the no-gap-intense sound responses. In initial test data, there were significant differences between gap and no-gap stimuli responses in all baseline-to-peak amplitudes, N1 ($t(24) = 5.53$, $p < 0.001$), P2 ($t(24) = -6.38$, $p < 0.001$), and N2 ($t(24) = 4.39$, $p < 0.001$). There were also significant differences in peak-to-peak amplitudes, N1-P2 ($t(24) = -8.06$, $p < 0.001$), P2-N2 ($t(24) = -8.27$, $p < 0.001$). Similarly, in retest data, there were significant differences in all baseline-to-peak and peak-to-peak amplitudes between gap and no-gap stimuli responses (N1 ($t(25) = 5.78$, $p < 0.001$), P2 ($t(25) = -6.55$, $p < 0.001$), N2 ($t(25) = 3.59$, $p < 0.001$), N1-P2 ($t(25) = -8.32$, $p < 0.001$), P2-N2 ($t(25) = -6.08$, $p < 0.001$)). Pearson's correlations used for test-retest reliability assessment of ALR peak amplitudes ranged from 0.28 to 0.57 in the gap condition and from 0.64 to 0.87 in the no-gap condition (Table 3.1). No-gap-intense sound responses showed greater significant correlations than those of the gap-intense sound responses in all indices. In particular, two peak-to-peak amplitudes in no-gap-intense sound responses were considered highly correlated.

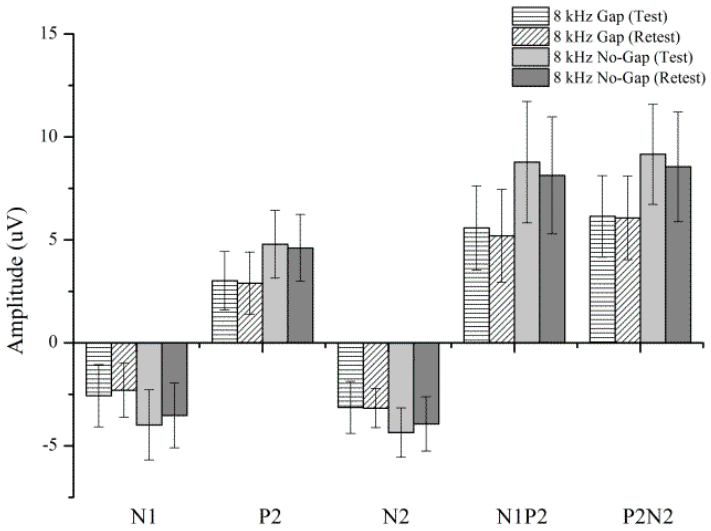

Figure 3.3 ALR peak amplitudes in response to gap-intense and no-gap-intense auditory stimuli with 8 kHz background noise (test and retest). No-gap-intense sound responses were greater than gap-intense sound responses in both sessions, and significant differences ($p < 0.001$) were found across all indices. There were no significant differences in mean amplitudes between the test and the retest.

Table 3.1 Pearson's correlations with *p*-values between the test and retest sessions

	8 kHz background noise		600 Hz background noise	
	Pearson's correlation coefficient	*p*-value	Pearson's correlation coefficient	*p*-value
Gap-intense sound response				
N1	0.383	0.059	0.487	0.025
P2	0.363	0.074	0.336	0.136
N2	0.283	0.170	0.721	< 0.001
N1-P2	0.568	0.003	0.419	0.059
P2-N2	0.478	0.016	0.567	0.007
No-gap-intense sound response				
N1	0.801	< 0.001	0.667	0.001
P2	0.791	< 0.001	0.513	0.017
N2	0.644	0.001	0.779	< 0.001
N1-P2	0.873	< 0.001	0.757	< 0.001
P2-N2	0.816	< 0.001	0.769	< 0.001

At the 600 Hz background noise, the overall gap-intense sound responses showed smaller magnitudes than those of no-gap-intense sound responses, as shown in Figure 3.4. In initial test data, there were significant differences in all indices between gap- and no-gap-intense sound responses (N1 ($t(22) = 4.40$, $p < 0.001$), P2 ($t(22) = -8.77$, $p < 0.001$), and N2 ($t(22) = 3.78$, $p < 0.001$), N1-P2 ($t(22) = -8.75$, $p < 0.001$), P2-N2 ($t(22) = -7.02$, $p < 0.001$)). Similarly, retest data revealed that there were significant differences in all baseline-to-peak and peak-to-peak amplitudes between gap and no-gap stimuli responses (N1 ($t(23) = 5.58$, $p < 0.001$), P2 ($t(23) = -7.38$, $p < 0.001$), N2 ($t(23) = 5.67$, $p < 0.001$), N1-P2 ($t(23) = -8.24$, $p < 0.001$), P2-N2 ($t(23) = -7.42$, $p < 0.001$)). Pearson's correlations of ALR peak amplitudes between the

test and retest ranged from 0.34 to 0.72 in the gap condition and from 0.51 to 0.78 in the no-gap condition. With a 600 Hz frequency background noise, no-gap-intense sound responses showed greater significant correlations than those of the gap-intense sound responses in all indices. Similarly, two peak-to-peak amplitudes in no-gap-intense sound responses were also considered highly correlated.

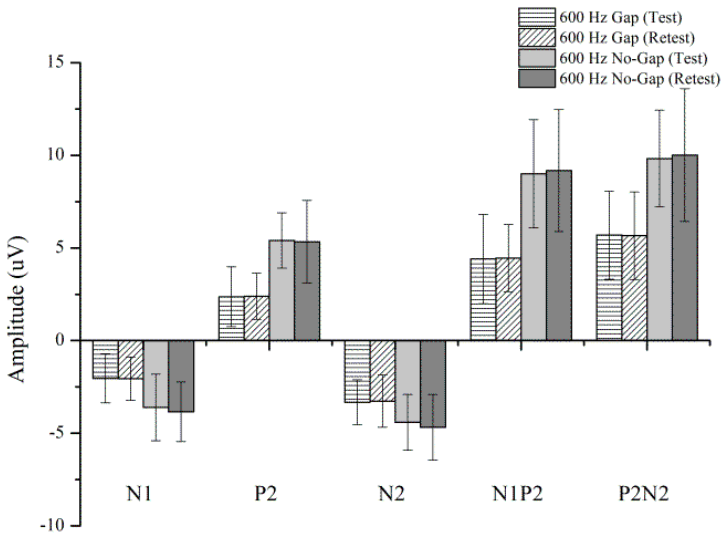

Figure 3.4 ALR peak amplitudes in response to gap-intense and no-gap-intense auditory stimuli with 600 Hz background noise (test and retest). Similarly to the results found using 8 kHz background noise, no-gap-intense sound responses were greater than gap-intense sound responses in both sessions, and significant differences ($p < 0.001$) were found across all indices. There were no significant differences in mean amplitudes between the test and

the retest. Note the larger differences in P2 compared with the results with 8 kHz background noise.

3.3.2. Gap-Prepulse Inhibition (GPI) Ratios

As shown in Figure 3.5, overall GPI mean ratios of N1, P2, N2, N1-P2, and P2-N2 were below 1.0 in the test and retest sessions. No significant three-way interactions were detected among sessions, background noise frequencies, or gap presence for all baseline-to-peak amplitudes and peak-to-peak amplitudes. A main effect for gap presence was found in all indices (N1 ($F(1,20) = 61.4$, $p < 0.001$), P2 ($F(1,20) = 81.6$, $p < 0.001$), N2 ($F(1,20) = 43.6$, $p < 0.001$), N1-P2 ($F(1,20) = 97.2$, $p < 0.001$), P2-N2 ($F(1,20) = 74.1$, $p < 0.001$)). Significant two-way interactions between background frequencies and gap presence were found in P2, N1-P2, and P2-N2 (P2 ($F(1,20) = 13.4$, $p = 0.002$), N1-P2 ($F(1,20) = 13.7$, $p = 0.001$), P2-N2 ($F(1,20) = 22.1$, $p = 0.023$)). Using the test and retest data at 8 kHz and 600 Hz of background noise, the one-tailed one-sample *t*-test confirmed significant inhibitions for all GPI indices (Table 3.2). In baseline-to-peak amplitudes, P2 showed lower mean values with higher absolute t-values than N1 and N2, especially with low background noise. The GPI ratios of peak-to-peak amplitudes showed relatively higher absolute t-values than those of baseline-to-peak amplitudes, and the overall highest absolute t-values were found in the N1-P2 amplitude.

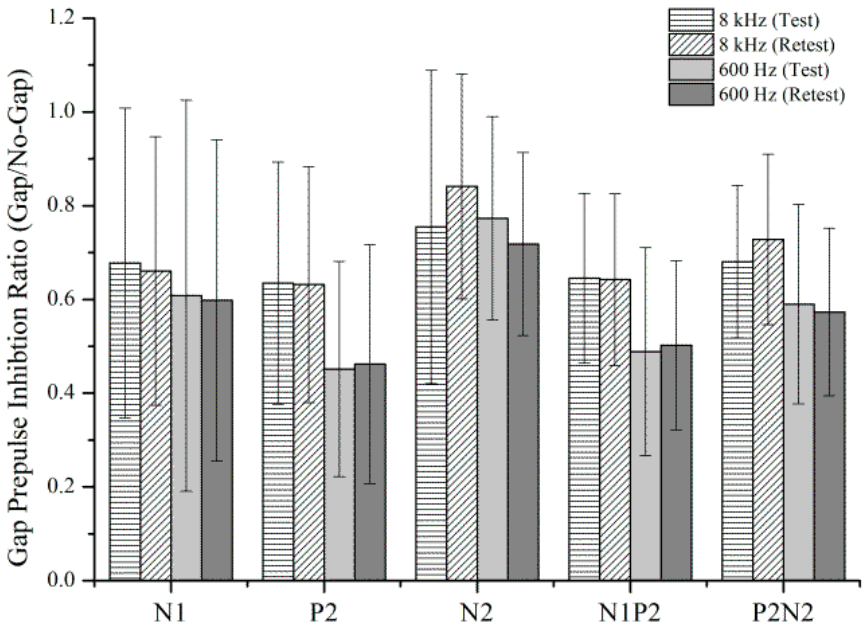

Figure 3.5 Gap-prepulse inhibition ratios of typical ALR peaks for each high and low background frequency (test and retest). A lower ratio indicates greater inhibition by the preceded temporal gap. Overall mean ratios of all indices were below 1.0, where the value 1.0 indicates no difference between gap-intense and no-gap-intense sound responses. Significant differences were found across all indices in the one-tailed one-sample *t*-test. Note that P2 and N1-P2 showed similar lower inhibition ratios compared to the other indices, but the overall highest absolute t-values were found in N1-P2.

Table 3.2 One-tailed one-sample *t*-test of gap-prepulse inhibition (GPI) ratios

	8 kHz background noise		600 Hz background noise	
	Test	Retest	Test	Retest
N1	$t(24)=-4.881^{b}$	$t(25)=-6.039^{b}$	$t(22)=-4.490^{b}$	$t(23)=-5.748^{b}$
P2	$t(24)=-7.079^{b}$	$t(25)=-7.467^{b}$	$t(22)=-11.455^{b}$	$t(23)=-10.338^{b}$
N2	$t(24)=-3.664^{a}$	$t(25)=-3.369^{a}$	$t(22)=-5.027^{b}$	$t(23)=-7.071^{b}$
N1-P2	$t(24)=-9.805^{b}$	$t(25)=-9.949^{b}$	$t(22)=-11.056^{b}$	$t(23)=-13.514^{b}$
P2-N2	$t(24)=-9.821^{b}$	$t(25)=-7.614^{b}$	$t(22)=-9.236^{b}$	$t(23)=-11.696^{b}$

$^{a}p < .01$, $^{b}p < .001$

3.3.3. Cumulative Average Trend Analysis

Figure 3.6 shows the variations in the N1-P2 and P2-N2 GPI ratios with trial numbers from the beginning to the end of an experiment. In each index, an initial gradual decline in the GPI ratio in response to repeated intense auditory stimuli was observed. In the N1-P2 GPI ratios, a significant difference was found in the overall ANOVA result ($F(4, 104) = 24.9$, $p < 0.001$). The mean P2-N2 GPI ratios also differed significantly for trial numbers ($F(3, 74) = 31.3$, $p < 0.001$). Compared with the final averaged values in the overall averaged data of both ratios, the reduction rates were below 5 % after 100 trials, and the pairwise comparisons confirmed that no significant differences were found in later trials for both N1-P2 and P2-N2 GPI ratios.

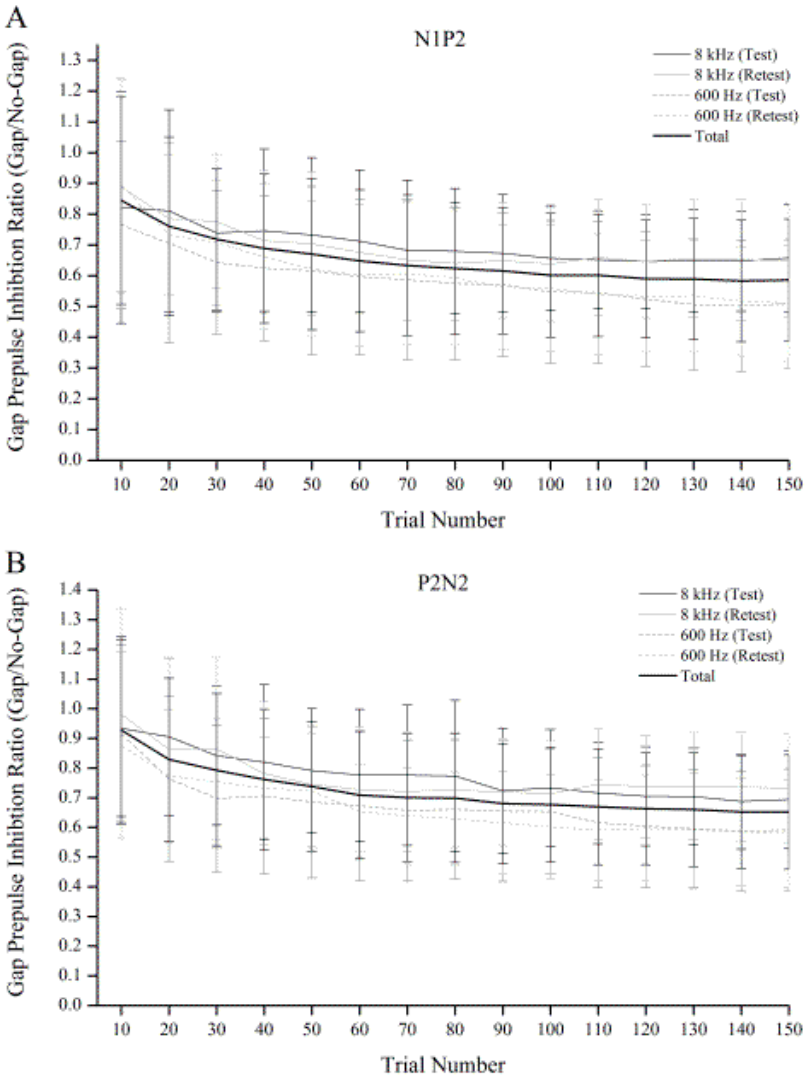

Figure 3.6 Variations in N1-P2 (A) and P2-N2 (B) GPI ratios with stimulus repetitions (test and retest). Note that an initial decline was shown in each index, and this decrease was nearly complete after approximately 100 stimulus repetitions.

3.3.4. Stimulus Parameter Analysis

Figure 3.7 shows the GPI ratios in various stimuli conditions. The mean N1-P2 GPI ratios differed significantly for different gap lengths ($F(2, 13) = 10.4$, $p = 0.002$). The mean P2-N2 GPI ratios also differed significantly between different gap lengths ($F(2, 14) = 6.9$, $p = 0.006$). The longer gap length elicited the smaller mean N1-P2 and P2-N2 GPI ratios. Post hoc pairwise comparison tests using Bonferroni's correction showed that the difference in the N1-P2 GPI ratio between the 20 ms and 200 ms gap lengths was significant ($p = 0.009$). A significant difference was found between the 20 ms and 200 ms gap lengths in the P2-N2 GPI ratio as well ($p = 0.004$). The effect of ISI on the N1-P2 GPI ratio was also significant ($F(2, 10) = 10.2$, $p = 0.005$), and the significant difference was only found between 20 ms and 100 ms ISI lengths ($p = 0.001$). However, the effect of ISI on the P2-N2 GPI ratio was not significant ($F(2, 11) = 3.1$, $p = 0.086$). There were no significant effects of the background amplitudes on either the N1-P2 ($p = 0.177$) or the P2-N2 ($p = 0.237$) GPI ratios.

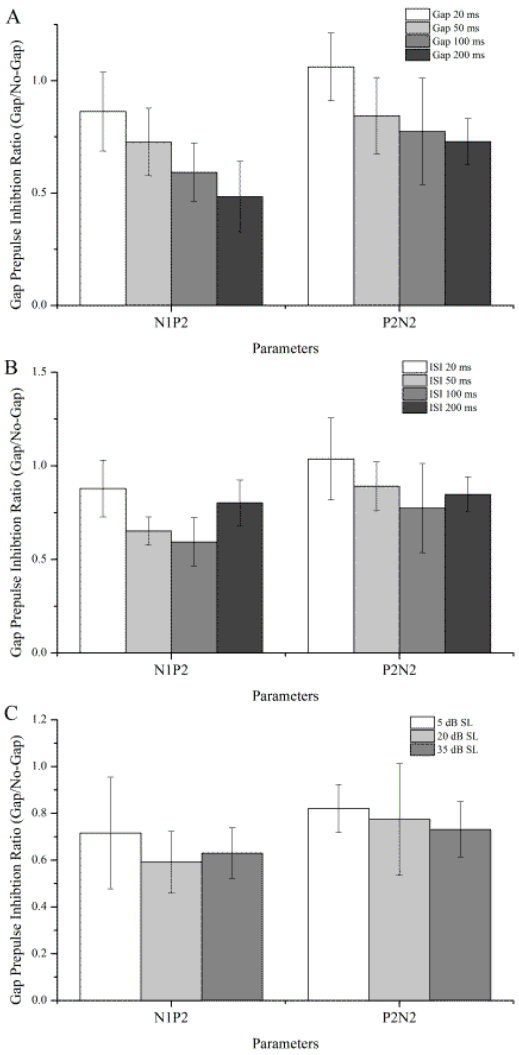

Figure 3.7 Changes in N1-P2 and P2-N2 GPI ratios under different gap (A), ISI (B), and background intensity (C) conditions. Four different gap durations (20, 50, 100, and 200 ms), four different ISI values (20, 50, 100, and 200 ms), and three different background intensity conditions (5, 20, and 35 dB SL) were tested. The main effects of gap duration and ISI on N1-P2 GPI ratios were identified in the one-way ANOVA with repeated measures.

3.4. Discussion

3.4.1. GPI of the ALR

The primary aim of this study was to find the inhibitions of ALR typical peaks in response to the gap-intense sound paradigm in normal hearing subjects. Consistent with the hypothesis, significant inhibitions of gap-prepulse stimuli were found in N1, P2, and N2. Thus, the response to the intense auditory stimulus was attenuated to some degree if a temporal gap prior to an intense auditory stimulus was recognized in the auditory cortex of the brain. The results from the analyses are consistent with those of previous studies that found evidence for the inhibitions of cortical responses caused by a weaker pre-pulse. In terms of temporal gap recognition in the auditory cortex, these findings are comparable with the results of earlier studies that reported that ALR was elicited in response to temporal gaps in noise (68, 72-78). The relatively large baseline fluctuation in this study, even after baseline correction, could be explained by the effect of the AEP in response to the pre-stimuli, such as the temporal gap and the background noise. The results showed that overall test-retest reliability of no-gap-intense sound responses was greater than that of gap-intense sound responses. This result was most likely caused by fluctuation of the baseline due to pre-stimulus gaps.

Concerning the GPI ratios of the baseline-to-peak amplitudes, the P2 component showed relatively greater inhibition than the N1 and N2 components, especially at a low-frequency background noise. This

observation of background noise frequency agrees with Fournier and Hebert's study, which measured an electromyogram of eye blinks in the gap-startle paradigm. In their research, the control group showed relatively lower inhibition at high- compared to low-frequency background noise, although they used a broader frequency bandwidth (1000 Hz) for the background noise. A pure tone noise was used in this study. However, the effect of the background frequency in the gap-intense sound paradigm in humans is still unclear because the requisite threshold of the gap duration for gap perception or ALR measurement increases with lower background center frequency (76, 79, 80). Relatively larger variation in the correlation coefficients of the N2 component (Table 3.1), especially in the gap-intense sound response, might originate from the evidence that the N2 component reflects cognitive processes, such as the assessment of stimuli and recognition memory, whereas early N1 and P2 components are more related to pre-attentional and automatic sensory events (81).

In regard to the comparison between baseline-to-peak and peak-to-peak components, peak-to-peak amplitudes showed more significant inhibition, and this result was also likely due to the pre-stimuli-evoked potential as mentioned above. Taken together, these data suggested that the N1-P2 complex is the best index to reflect GPI of ALR in the gap-intense sound paradigm in humans. In practice, the N1-P2 complex has been applied to investigate auditory plasticity (7, 18).

3.4.2. Minimum Required Trial Number

To my knowledge, no study has investigated the GPI ratio change of ALR in the gap-intense sound paradigm in humans. Determining the minimum required trial number using a GPI trend analysis is very important, especially in the gap-intense sound paradigm, because the interval between the intense auditory stimuli should be longer than that of the general ALR measurement. The interval between intense auditory stimuli was pseudo-randomly set to 1 and 3 s in this study, and considering the average 2 s interval, the required time for one measurement was approximately 10 minutes (150 repetitions of each gap- and no-gap- intense auditory stimulus). In terms of clinical use of the gap- intense sound paradigm, it is clear that less test time is better to prevent subject drowsiness, which could lead to unreliable GPI ratios. When increasing trial numbers, both GPI ratios of N1-P2 and P2-N2 gradually declined, and then, after approximately 100 repetitions, the variations in the GPI ratios became stable. This result can be explained mainly by the signal-to-noise ratio, which is dependent on the experimental set-up, such as the noise levels of an acquisition device or a measurement environment and electrode-skin impedances. The signal-to-noise ratio of ALR is also related to the amplitudes of stimuli and the signal processing methods for averaging. In this study, a conventional ensemble averaging method was applied with only simple magnitude threshold rejection, which is generally used in the ALR test. The long-term habituation of the gap-intense sound response could be another reason for the initial decrease in the GPI ratio (82). The long-term habituation

of AEP is defined as the initial reduction of the AEP amplitude across blocks of auditory stimuli (83-87). This initial decrease in typical AEP peaks has been found in several previous studies. It can be proposed that at least 100 sweeps are necessary for healthy normal-hearing subjects to evaluate GPI ratios in the gap-intense sound paradigm with the ensemble averaging method. Further investigation of this number should be performed in an initial comparative study because it will increase due to the habituation deficit of ALR in tinnitus patients (88).

3.4.3. Effects of Stimulus Parameters on the GPI Ratio

Consistent with the hypothesis, the longer gap length elicited a higher GPI ratio. This finding corroborates the previous studies showing clearer ALR morphology or better gap detection ability in the longer temporal (68, 72, 77, 78). It is known that the ALR waveforms to gap offsets of relatively longer gaps (200-800 ms) have a distinct single-peaked N1 and that those to gap onsets of shorter (10-20 ms)- and longer- gaps have two negative peaks, N1a and N1b. The N1b peak is considered to be associated with the pre-attentive perception of the termination of an ongoing sound (72, 73). The clearer N1b was shown in ALR waveforms to gap onsets of the longer gaps and is possibly related to the lower GPI ratios with the longer gaps in the current study. This also might be because the offset of background noise (the onset of gap) acts as a pre-pulse event and because the subsequent onset of background noise (the offset of gap) acts as another pre-pulse event (89, 90). When the gap is long

enough, those two pre-pulse events may act as two separate stimuli making the inhibition stronger. Meanwhile, when the gap duration is insufficient, the effect of the two pre-pulse events may be mixed up. Thus, the relatively shorter gap functions as only a single pre-pulse event, which may reduce the effect of the inhibition to the intense sound. The temporal gaps primarily used in this study (100 ms duration) might evoke the N1a and N1b components of the previous studies however, those components were not observed prior to presentation of the intense sound. This might have been the case because the intensity and the single frequency of the background noise in the current study were ineffective compared to those of previous studies. Moreover, the neural refractory periods of the ALR to the gap onset and the intense sound might be insufficient due to the relatively short stimulus onset asynchrony condition of the current study (100 ms gap length for gap offsets and 1-3 s inter-trial intervals for gap onsets, respectively). Because a significant GPI was observed with the 100 ms gap duration in healthy subjects, a further comparative study would evaluate the degree of GPI with 100 ms or shorter gap duration. Regarding the ISI, the mean GPI ratio was lowest at 100 ms, which is consistent with the interval for the greatest suppression identified in earlier sensorimotor and sensory gating studies. In regards to background noise intensity, 5 dB SL was considered an adequate intensity to elicit the GPI of ALR. The results showed that gap duration and ISI are the primary GPI factors in determining stimuli parameters that will result in the greatest difference between healthy subjects and tinnitus patients. Moreover, careful

dB SL calibration of background noise intensity before every ALR measurement was suggested because temporal gaps in only 5 dB SL background noise can elicit considerable GPI ratios, and the loudness of the tinnitus often varies (91). As in several animal studies, dB SPL might be easier to apply because it does not need to reflect the tinnitus loudness or the hearing threshold. However, because tinnitus frequency is often matched with the frequency of hearing loss, it would be necessary to present auditory stimuli in dB SL in a human study. This point was also addressed in Fournier and Hebert's study (30).

3.5. Conclusion

This study found significant inhibition of typical ALR peaks in response to the gap-intense auditory stimuli in healthy normal-hearing subjects. The peak-to-peak amplitudes, especially the N1-P2 complex, reflected the GPI well in terms of suppression degree and test-retest reliability. Moreover, repetition with at least 100 stimuli in an ALR measurement was suggested as the minimum required trial number for a stable GPI ratio. Finally, the gap length and the ISI were the primary factors that affected the GPI ratios of the ALR. The findings of this study will be informative in future comparative studies of healthy subjects and tinnitus patients using the gap-intense sound paradigm with the ALR.

* Portions of this chapter were published in Psychophysiology Journal (Ku Y, Ahn JW, Kwon C, Suh MW, Lee JH, Oh SH, et al. Gap prepulse inhibition of the auditory late response in healthy subjects. Psychophysiology. 2015;52(11):1511-9.) (92)

Chapter 4. Clinical Application:

A Comparative Study

4.1. Introduction

Continuing the investigation of the hypothesis, a comparative study between tinnitus patients and age- and hearing loss-matched controls was performed using the information derived from a normative study. As a brief review of human studies of the GPI paradigm, a significant GPI deficit of patients with tinnitus was found in the response to 50-ms gap-embedded stimuli with both high- and low-frequency background noises, as compared with age- and hearing loss-matched controls (30). The authors pointed out that proof of the tinnitus filling in the gap hypothesis is still required. Some animal studies also reported the impairments of gap processing at various frequencies, including the tinnitus frequency of the animal model; they suggested that an impaired cortical processing caused the GPI deficit that occurred irrespective of the similarity between background frequency and tinnitus pitch in the patients with tinnitus. However, these two possible explanations might not be mutually exclusive because the group difference of gap inhibition was greater with high background frequency (closer to the tinnitus pitch). In another human study using the same ASR (93), no significant difference in the GPI at the gap of 100-ms duration was found between patients with tinnitus with moderate hearing loss on average and controls with normal hearing, possibly due to the

small number of study subjects. However, in their study, the amplitude of the ASR in patients with tinnitus having normal hearing was significantly greater than hearing loss-matched controls, consistent with the result of a previous study by Fournier and Hebert (30). If the tinnitus perfectly fills in the gap, the gap duration would not affect the gap inhibition deficit of patients with tinnitus. However, the GPI deficit of the ASR did not occur with the longer gap duration in the second human ASR study. If the tinnitus sound does not perfectly match with the background noise in terms of the pitch and loudness, the tinnitus sound is expected to partially fill in the gap. Thus, the gap could still act as the cue for the oncoming startle sound. We speculated that the longer gap would provide a better chance to compensate the effect of tinnitus by the top-down modulation such as the higher cortical processing (56). Since the shorter 50-ms gap might not be sufficiently long for the ASR modulation, the effect of tinnitus partially filling in the gap could hinder the gap processing in the ASR.

In one psychoacoustic gap detection test, the 50-ms gap duration was not significantly different between the patients with tinnitus and controls even with younger mean age and better hearing ability (45). When using shorter gap durations (< 30-ms), patients with tinnitus showed a similar detection ability as compared with age- and hearing loss-matched controls (31). The inconsistency between these results and those from studies of the ASR has two possible reasons. First, the discrepancy might be caused by the differences in related neural circuits between the ASR (subcortical pathway)

and auditory perception task (auditory and motor cortex). Second, higher cortical processing such as attention is involved in the psychoacoustic gap detection test, whereas the ASR is a pre-attentive response. Thus, attentive processing could compensate for subcortical inhibition deficits (31, 45).

The precise mechanism of tinnitus is not fully understood, but the most recent studies on tinnitus models indicate that tinnitus is a complex phenomenon that emerges from several malfunctioning neural circuits involving the auditory cortex and extra-auditory areas. In addition to reports on inhibitory mechanistic changes of the tonotopic frequency map in the auditory cortex (33, 39, 46, 47, 49, 94), the connectivity changes between auditory and extra-auditory areas have been detected in patients with tinnitus (95-97). Thus, it was hypothesized that the auditory late response (ALR), which reflects the processing of the auditory cortex, is an index of the GPI paradigm for the objective measure of tinnitus. In a recent review of the GPIAS, the necessity to assess the responses of the auditory cortex is highlighted (98). The N1-P2 complex, generated by the auditory cortex (7, 29, 81), is considered as a mainly obligatory response to sensory events, although it is possibly influenced by higher-level cognitive processing. Accordingly, the N1-P2 recording does not request the subject's attention, which is considered as a possible reason for the discrepancy between the ASR and psychoacoustic studies. Significant test-retest reliability of the N1-P2 complex in the measurement (7, 99, 100) or sensory gating (101) has been established. To my best knowledge, no study has investigated the effect

of tinnitus on the N1-P2 complex in the GPI paradigm. The first objective of the present study was to investigate whether the effect of tinnitus emerges on the N1-P2 complex in the GPI paradigm. The second objective was to address the tinnitus filling in the gap hypothesis if the effect of tinnitus was observed at the tinnitus-pitch-matched frequency background noise. Thus, the GPI degrees at the tinnitus-pitch-matched (8 kHz) frequency background noise were compared between patients with tinnitus and controls. Since inconsistent results were reported in the previous ASR studies with different gap durations, the comparisons with three different embedded gap durations (100-, 50-, and 20-ms) were performed. The GPI degrees at the tinnitus-pitch-non-matched frequency background noise were also assessed with the three gap durations. For the proof of the tinnitus filling in the gap concept, both groups should display significant gap inhibitions at the same gap condition, with the GPI deficit at the tinnitus-pitch-matched (8 kHz) frequency background noise. Additionally, the amplitudes of the N1-P2 complex without the effect of the gap-prepulse were compared, since enhanced ASR amplitudes of patients with tinnitus were reported in both of the previous ASR studies.

4.2. Methods

4.2.1. Subjects

Twenty patients with tinnitus were recruited at the Seoul National University Hospital after confirming tonal high-pitch (8 kHz) tinnitus in the tinnitus-matching test. In the tinnitus pitch- and loudness-matching test, patients were requested to choose the most similar sound with their perceived tinnitus among a pure tone, a narrowband noise, or a white noise. Next, a frequency adjustment from 0.125 to 8 kHz with the 1/1 octave step size was performed on patients who chose a pure tone or narrowband noise. To exclude the possibility of frequency confusion between neighboring octaves, the octave confusion test was performed at a frequency one octave higher than the first pitch-matched frequency and subsequently at a frequency one octave lower (102). After confirming the final octave-confirmed frequency, loudness was adjusted to identify the closest tinnitus loudness. On the day of an experiment, this matching procedure was repeated once before the ALR measurement. Subjects who could not confirm the tinnitus-matched background frequency in the subsequent test were excluded from the analysis (N = 2) and subjects who displayed unclear N1-P2 complex in their small baseline-to-peak amplitudes (< 1 μV) of the ALR were also excluded from the study (N = 2). Patients with tinnitus in this study perceived bilateral (N = 9) or unilateral (N = 7) continuous tinnitus for at least 6 months. Twenty age- and hearing loss-matched controls were recruited using poster ads or word of mouth. None of

the controls had suffered from tinnitus or any other neuropsychiatric diseases. Controls who displayed unclear N1-P2 complex were also excluded from the study (N = 2). For all subjects, hearing thresholds at six different octave frequencies (0.25, 0.5, 1, 2, 4, and 8 kHz) were measured by pure tone audiometry. The inclusion criteria of hearing thresholds at the 0.5, 1, and 8 kHz frequencies were 70, 30, and 70 dB HL, respectively, due to the maximum output limitation of the experimental equipment. Details of the subject characteristics are presented in Table 1. The independent sample t-test confirmed no significant differences in the age and hearing threshold at each frequency between groups. This study was approved by the Institutional Review Board of Seoul National University Hospital, and all subjects provided written consent.

Table 4.1 Subject Characteristics

	Tinnitus (N = 16)	Controls (N = 18)	*p*-value
Age			
Years (SD)	59.2 (8)	59.2 (6)	.991
Range	42 to 72	47 to 72	
Gender			
Male	10	11	
Female	6	7	
Hearing Threshold (dB HL)			
8 kHz (SD)	39.1 (17.1)	34.4 (18.8)	.461
600 Hz (SD)	13.1 (8.5)	16.1 (11.2)	.383
1 kHz (SD)	17.4 (8.2)	17.2 (8.9)	.943

SD, standard deviation

4.2.2. Stimuli

Similarly with a normative study, the stimulus contained a continuous pure tone background noise and an intense auditory stimulus in the dB SL scale to reflect the hearing threshold of each subject. Background noises were set to 20 dB SL for both 8 kHz and 600 Hz frequencies. When applying the 600 Hz-frequency background noise, the hearing threshold at 500 Hz, which is the closest test frequency to 600 Hz, was used. The intense auditory stimulus was a 1-kHz tone burst of 20-ms duration, including 5 cycles each of the rise and fall times. The intensity of the intense sound was 65 dB SL. In the gap-intense auditory stimulus, the inter-stimulus interval (ISI) between the offset of a temporal gap and the onset of intense auditory stimulus was set to 100 ms, which is very close to the optimal duration for sensorimotor- and sensory-gating. Because significant GPI of the N1-P2 complex was found at the 100-ms gap duration in young and normal hearing subjects, temporal gaps with shorter durations (50 and 20 ms) and the 100-ms duration were investigated. The inter-trial intervals (ITI) between the intense auditory stimuli were pseudorandomly altered between 1 and 3 s to minimize the subject's prediction. The orders of gap-intense auditory stimuli and no-gap-intense auditory stimuli were also pseudorandomly generated. The number of trials for each type of auditory stimulus was 100 each, and thus, the required time of one ALR measurement was less than approximately 10 minutes. All stimuli were presented by the developed research platform to perform the GPI paradigm with the ER-2 insert earphone, which showed a flat high-frequency

response. All sound output at seven different frequencies (0.2, 0.5, 1, 2, 4, 6.3, and 8 kHz) was calibrated by a sound level meter (Type 2250 Sound Level Meter, Bruel & Kjaer, Nærum, Denmark) with an ear simulator simulator (IEC Ear Simulator RA0045, G.R.A.S. Sound & Vibration, Holte, Denmark) in a soundproof booth. The maximum intensity of the intense sound was 95 dB HL and the intensity of the background noise was up to 90 dB HL depending on the hearing threshold of each subject. According to the calibration result, the maximum intensities in the equivalent dB SPL were 104, 103, and 102 dB SPL at 1 kHz, 8 kHz, and 500 Hz, respectively. These maximum intensities were lower than the maximum undistorted outputs (< 3% third harmonic distortion) of the ER-2 insert earphone, which were 108, 112, and 108 dB SPL at 1 kHz, 8 kHz, and 500 Hz, respectively.

4.2.3. ALR Recording

In a soundproof booth, subjects were seated in a reclining armchair and requested to refrain from moving too much, closing their eyes, and falling asleep. They were also instructed not to pay attention to the stimuli (passive listening) and to watch the muted video clips, which were used to deviate participants' attention away from the auditory stimuli and alleviate drowsiness. The video clips were the text-based videos for the education of history, science, or medicine, and the materials considered as highly emotional were excluded. The brightness of the monitor was kept relatively dim (30% of

maximum brightness level). The measurement started with skin preparation to lower the skin-electrode contact impedance below 5 kΩ. The impedance differences between electrodes were maintained at < 2 kΩ. A 10-mm gold disc electrode (F-E5GH, Grass Technologies, Warwick, RI) with conductive paste was located on the vertex (Cz) for the active electrode following the guidelines of the ALR measurement (1). Disposable and adhesive silver-silver chloride electrodes (KendallTM 100 series, Covidien llc, Mansfield, MA) were placed on the ipsilateral mastoid (A1 or A2) and forehead (Fpz), serving as a reference electrode and ground electrode, respectively. The total 700-ms epoch including the 100-ms pre-stimulus time for the baseline correction were recorded. An analog band-pass filter with a cutoff frequency of 1 to 100 Hz was applied before the ADC, and the sampling rate of ADC was 1 kHz. The digital low-pass Butterworth filter with a 30-Hz cutoff frequency was applied to the epoch for smoothing waveforms. In total, 100 epochs for each gap-intense auditory stimulus and no-gap-intense auditory stimulus were recorded, and epochs greater than ± 50 μV were excluded (< 10% in all measurements). In one measurement, two ALR waveforms in response to the gap-intense auditory stimuli and no-gap-intense auditory stimuli, respectively, were acquired by the ensemble averaging of epochs. At each gap duration, the ALR was recorded in response to the auditory stimuli of the GPI paradigm with the first background frequency. After a 3-min break, the ALR to the auditory stimuli with the second background frequency was recorded, and thus, a total of 6 ALR measurements were performed on each subject. A break longer than

3 min between each measurement was allowed for subjects who showed drowsiness or fatigue. The orders of gap durations and background frequencies were counterbalanced in each group to prevent the effect of habituation on the amplitude or inhibition of the ALR. The order of 100-, 50-, and 20-ms gap durations was circularly shifted by one subject for each group; concurrently the order of 8 kHz and 600 Hz background noises was reversed by one subject.

4.2.4. Data Analysis

The amplitudes of the N1-P2 complex were computed by a peak detection algorithm in a predefined latency region for each peak (N1: 60–180 ms, P2: 100–250 ms). A negative peak, N1, was generally found between 90 and 150 ms from the onset of the intense auditory stimulus. A positive peak, P2, was found in the 160 to 200 ms, followed by a second negative peak. The peak detection was confirmed by a visual inspection. The GPI ratio was defined as the reduction ratio of the amplitudes (GPI = Gap/No-Gap), in which Gap represents the peak-to-peak amplitude of the N1-P2 complex in response to the gap-intense auditory stimuli, whereas No-Gap represents the peak-to-peak amplitude of the N1-P2 complex in response to the no-gap-intense auditory stimuli.

First, time-dependent changes for the No-gap response were analyzed to address the short-term (across trials) and long-term (across measurements) habituation effects for each group in the present study. No-Gap responses

were used to exclude the effect of the gap-prepulse. For the short-term habituation, 60 trials were used for the averaging of No-Gap responses to provide sufficient quality of signal-to-noise ratios. The start point for the averaging was increased by the step size of two trials. The amplitudes of total 6 measurements per one subject were averaged. One-way analysis of variance (ANOVA) with repeated measures was performed to assess the changes across trials for each group. For the long-term habituation, one-way ANOVAs with repeated measures were conducted on the amplitude of each measurement for each group in two different sequential and counterbalanced orders, respectively. Second, a two-way mixed repeated-measures ANOVA with group (tinnitus vs. controls) as a between-subjects factor and background frequency (8 kHz vs. 600 Hz) as a within-subjects factor was performed to address the group difference. For the GPI of the N1-P2 complex, a 2 × (3 × 2) mixed ANOVA was used with group (tinnitus vs. controls) as a between-subjects factor and gap duration (100-, 50-, and 20-ms) and background frequency (8 kHz vs. 600 Hz) as within-subjects factors.

Further analysis to address the gender difference was performed for each group because it is known that female individuals generally show less pre-pulse inhibition (PPI) than male individuals (103, 104). For the amplitude of the N1-P2 complex in each group, a two-way mixed repeated-measures ANOVA with gender (female vs. male) as a between-subjects factor and background frequency (8 kHz vs. 600 Hz) as a within-subjects factor was performed. For the GPI in each group, a 2 × (3 × 2) mixed ANOVA was used

with gender (female vs. male) as a between-subjects factor and gap duration (100-, 50-, and 20-ms) and background frequency (8 kHz vs. 600 Hz) as within-subjects factors.

The Greenhouse–Geisser correction was adopted in the cases of violation of sphericity. Follow-up analysis using ANOVA or independent sample *t*-test was run on significant interactions. One sample *t*-tests relative to 1.0, which indicates no difference, were performed for post hoc comparisons to determine whether the GPI ratios of each group were significant.

4.3. Results

Figure 4.1 and Figure 4.2 display the grand averaging waveforms of the two groups. At each condition, clear N1-P2 complex was shown between 60 and 200 ms from the onset of the intense auditory stimulus. In the controls, the effect of the short-term habituation was not significant, $F < 1$. The mean amplitudes in response to first and last 60 trials were 9.51 μV (SD: 3.39) and 9.24 μV (SD: 3.41), respectively. In the tinnitus group, no significant effect of the short-term habituation was found , $F < 1$ and the mean amplitudes in response to first and last 60 trials were 8.17 μV (SD: 2.90) and 7.67 μV (SD: 2.44), respectively. For the long-term habituation, in the controls, the effect of measurements in sequential order was significant, $F(5,85) = 12.4$, $p < .001$, $\eta^2 = .42$. The mean amplitudes in response to first and last measurements were 10.26 μV (SD: 3.64) and 7.95 μV (SD: 3.05), respectively. However, in the counterbalanced order by gap durations and background frequencies, no significant effect was found, $F < 1$. The maximum mean amplitude was 9.34 μV (SD: 3.39) at the 100-ms gap duration with the 600 Hz-frequency background noise; and the minimum mean amplitude was 8.73 μV (SD: 2.73) at the 20-ms gap duration with the 8 kHz-frequency background noise. The effect of measurements in sequential order was also significant in the tinnitus group, $F(5,75) = 4.7$, $p = .001$, $\eta^2 = .24$. The mean amplitudes in response to first and last measurements were 8.96 μV (SD: 2.83) and 6.66 μV (SD: 2.03), respectively. However, no significant effect was found in the counterbalanced order, $F < 1$. The maximum mean amplitude was 7.84 μV (SD: 3.47) at the

20-ms gap duration with the 600 Hz-frequency background noise; and the minimum mean amplitude was 7.38 μV (SD: 2.56) at the 50-ms gap duration with the 600 Hz-frequency background noise.

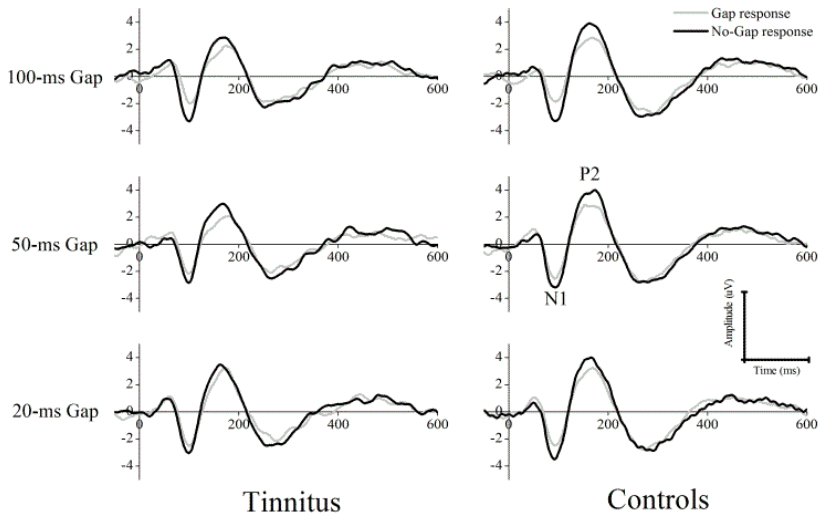

Figure 4.1 Grand averaging waveforms of the auditory late response (ALR) on vertex (Cz) in response to gap- and no-gap-intense auditory stimuli with the 8 kHz-frequency background noise.

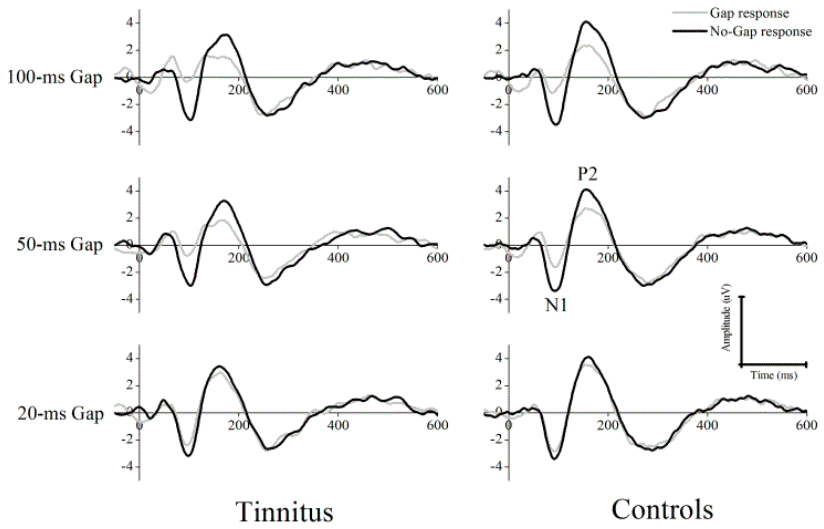

Figure 4.2 Grand averaging waveforms of the auditory late response (ALR) on vertex (Cz) in response to gap- and no-gap-intense auditory stimuli with the 600 Hz-frequency background noise.

As shown in Figure 4.3 and Figure 4.4, at the No-Gap condition (i.e., no effect of the gap-prepulse), tinnitus group displayed the tendency to have less amplitude of the N1-P2 complex than controls, but no group effect was found, $F(1,31) = 2.4$, $p = .132$. There was no main effect of background frequency, $F < 1$. Figure 4.5 shows the GPI ratios of the N1-P2 complex in 8 kHz- and 600 Hz-frequency background noises. A lower ratio indicates greater inhibition by the inserted temporal gap. A mixed ANOVA showed a main effect of gap duration, $F(2,62) = 17.8$, $p < .001$, $\eta^2 = .37$. The three-way interaction between group, gap duration, and background frequency was not significant,

$F(2,62) = 2.5$, $p = .091$. There was a significant interaction between gap duration and background frequency, $F(2,62) = 5.9$, $p = .004$, $\eta^2 = .16$. A 2 × 3 mixed ANOVA with group (tinnitus vs. controls) as a between-subjects factor and gap duration (100, 50, and 20 ms) as a within-subjects factor was conducted on each background frequency to address the effect of gap duration on the GPI ratios separately. For the GPI ratio with the 8 kHz-frequency background noise, there was a significant interaction between group and gap duration, $F(2,64) = 4.9$, $p = .010$, $\eta^2 = .13$. For the follow-up of this interaction, an independent sample *t*-test for each gap duration was performed. At the 20-ms gap condition, there was a significant difference between tinnitus group and controls, $t(32) = -3.49$, $p = .001$, with means of 0.99 (SD: 0.21) and 0.76 (SD: 0.18), respectively. At other gap durations, the differences between the two groups were not significant. There was no significant main effect of gap duration, $F(2,64) = 1.4$, $p = .242$. In contrast, with the 600 Hz-frequency background noise, a main effect of gap duration was found, $F(2,62) = 20.2$, $p < .001$, $\eta^2 = .39$, but there was no significant interaction between group and gap duration. However, the follow-up independent sample *t*-test at the 20-ms gap duration, in which the group difference was found with the 8 kHz-frequency background noise, was performed to compare the effect of the background frequency on the GPI ratio. At the 20-ms gap duration, no significant difference was found between the tinnitus group and controls, $t(32) = .62$, $p = .541$, with means of 0.94 (SD: 0.23) and 0.89 (SD: 0.24), respectively.

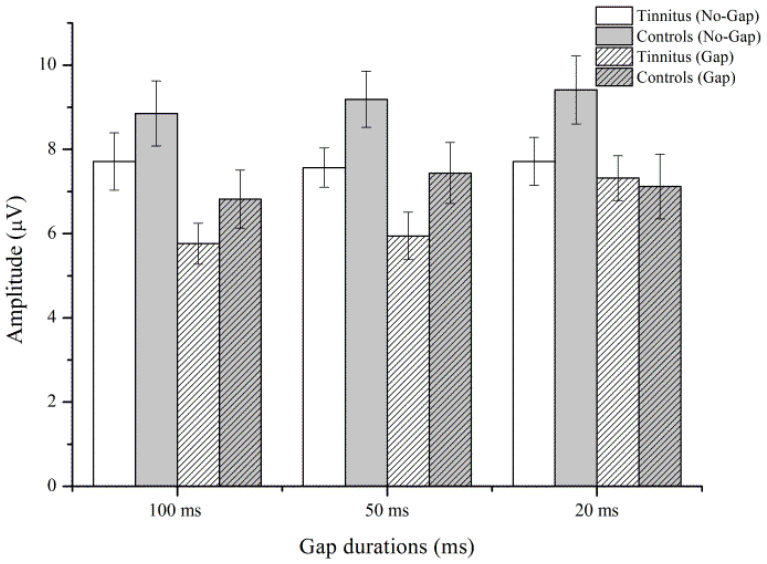

Figure 4.3 Peak-to-peak amplitudes (standard error of mean, SEM) of the N1-P2 complex in response to gap- and no-gap-intense auditory stimuli with the 8 kHz-frequency background noise. With the observation on no-gap responses, tinnitus group displayed the tendency to have less amplitude than controls, but no group effect was found.

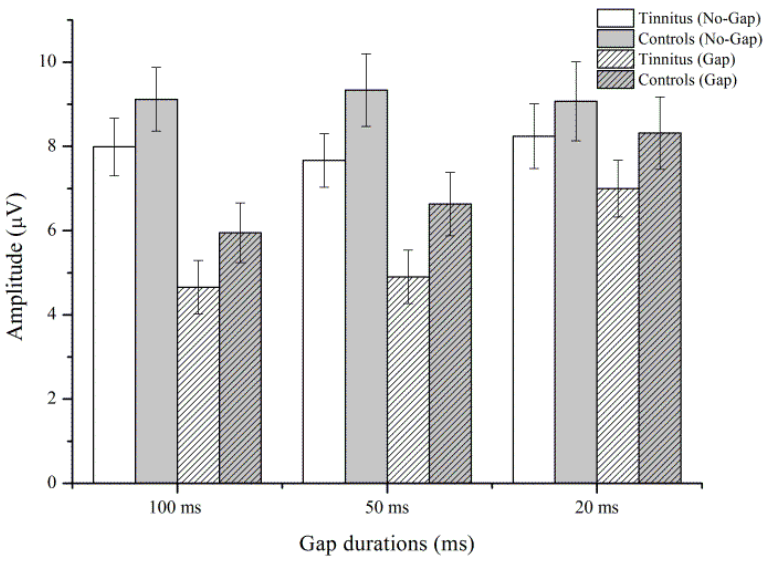

Figure 4.4 Peak-to-peak amplitudes (standard error of mean, SEM) of the N1-P2 complex in response to gap- and no-gap-intense stimuli with the 600 Hz-frequency background noise. Similarly with the 8 kHz-frequency background noise, tinnitus group displayed the tendency to have less amplitude than controls, but no group effect was found.

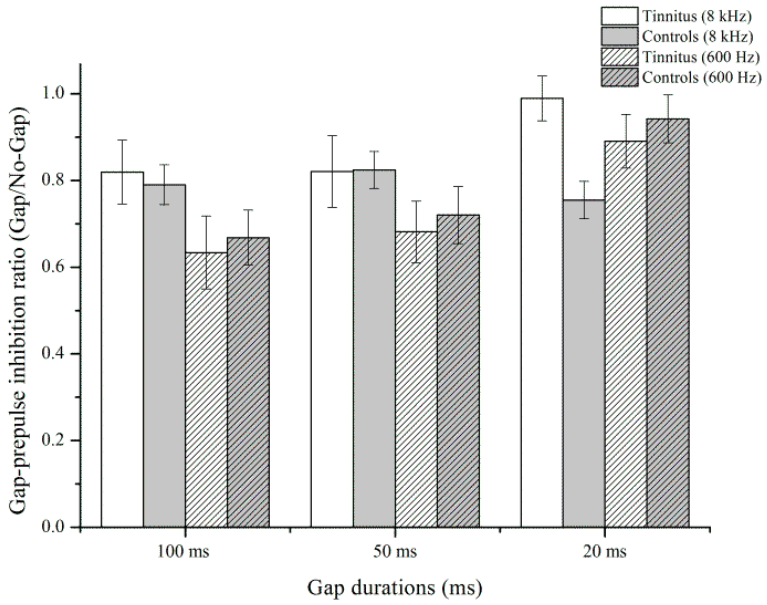

Figure 4.5 Gap-prepulse inhibition ratios (standard error of mean, SEM) of the N1-P2 complex with 8 kHz- and 600 Hz-frequency background noises. A lower ratio indicates greater inhibition by the temporal gap. A group difference was found only at the 20-ms gap duration with 8 kHz-frequency background noise.

In the one sample *t*-tests, at the 20-ms gap duration with the 8 kHz-frequency background noise that showed group-wise difference, significant inhibition was found only in the controls, $t(17) = -5.66$, $p < .001$, but not in the tinnitus group, $t(15) = -.20$, $p = .841$. No significant inhibition was observed in both groups at the 20-ms gap duration with the 600 Hz-frequency background noise. Significant inhibitions were observed in both groups under all other conditions (Table 4.2).

Table 4.2 One sample *t*-tests of gap-prepulse inhibition (GPI) ratios

	8 kHz-frequency background noise		600 Hz-frequency background noise	
Gap duration	Tinnitus	Controls	Tinnitus	Controls
20 ms	*.841*	***<.001***	*.098*	*.309*
50 ms	***.047***	***.001***	***<.001***	***.001***
100 ms	***.027***	***<.001***	***.001***	***<.001***

Note. The bold *p*-values represent the cases where the GPI ratios were significantly lower than 1.0.

For the analysis of the gender difference, in the controls, a main effect of gender on the amplitude of the N1-P2 complex was not significant, $F < 1$. Overall mean N1-P2 amplitudes of females and males were 10.15 μV (SD: 3.21) and 8.52 μV (SD: 3.21), respectively. Interestingly, women showed greater amplitudes than men in all 6 measurements, except in the tinnitus group. In the tinnitus group, similarly, a main effect of gender was not significant, $F < 1$. Overall mean N1-P2 amplitudes of females and males were 8.21 μV (SD: 2.71) and 7.58 μV (SD: 2.28), respectively. For the GPI, in the controls, although the mixed ANOVA showed a main effect of gap duration, $F(2,30) = 11.5$, $p < .001$, $\eta^2 = .43$, a main effect of gender was not significant, $F < 1$. Overall mean GPI degrees of females and males in the controls were 0.81 (SD: 0.21) and 0.76 (SD: 0.25), respectively. In the tinnitus group, similarly, a main effect of gap duration was observed, $F(2,28) = 10.5$, $p < .001$, $\eta^2 = .43$ but there was no main effect of gender, $F < 1$. Overall, mean GPI degrees of females and males in the tinnitus group were 0.82 (SD: 0.34) and 0.79 (SD: 0.27), respectively.

4.4. Discussion

4.4.1. Comparisons with Other Human GPI Studies

The first main finding of the present study was that the GPI deficit of patients with tinnitus was found on the N1-P2 complex with the tinnitus-pitch-matched frequency background noise. The deficit did not emerge at the 100- and 50-ms gap durations, but at the 20-ms gap duration. Thus, the gap duration affected the GPI deficit of patients with tinnitus. The second main finding was that both patients with tinnitus and controls showed the GPI deficit at the 20-ms gap duration with the tinnitus-non-pitch-matched frequency background noise. Thus, the deficits with the low-frequency background noise might not be due to the presence of tinnitus. The relatively short gap could have a lower inhibitory effect on the N1-P2 complex with low-frequency background noise than with high-frequency background noise. Each of these findings is discussed below.

First, the current findings of the present study at three different gap durations were compared with previous human studies of the ASR and gap detection ability. The effect of different gap durations on the GPI could be interpreted in two aspects. First, the cue onset timing for the incoming intense sound could affect the GPI of the N1-P2 complex. PPI could be subjected to top-down modulation by higher level processes such as attention or cognition (69, 105). The shorter cue onset timing would apparently allow less time for the higher cortical processing. The cue timing depends on the onset of gap as well as the

offset of gap (90, 106). In the present study, the duration between the offset of gap and the onset of intense sound (i.e. ISI) was fixed to 100 ms. Accordingly, shorter gap duration provided shorter cue onset timing, which is the duration between the onset of gap and the onset of intense stimulus. Second, the duration between the onset and offset of gap also could affect the GPI, especially for cases in which the gap is not completely filled by tinnitus sound. For the ALRs to gaps in noise, two separate responses to gap onset and offset occurred with long gap durations (200-800 ms). With shorter gap durations up to 20 ms, the amplitude of N1-P2 complex in one ALR increased as the gap duration increased (72). Thus, when the gap duration is sufficiently long, two cue events could contribute independently to the inhibition. However, if the gap duration is not sufficiently long to be perceived as two separate cues, this relatively shorter gap would act as only one cue event and possibly lead to weaker inhibition.

At 100- and 50-ms gap durations, the N1-P2 complexes of both groups were significantly inhibited by the gap-prepulse with both 8 kHz- and 600 Hz-frequency background noises, without group-wise difference. Thus, the gap might not be totally masked by tinnitus sound. Even if the tinnitus partially fills in the gap, the provided cue onset timings and gap durations are sufficiently long to allow higher cortical processing even for patients with tinnitus. The result of the previous ASR study with the 100-ms gap duration (with no ISI information) was consistent, which showed that there were no differences in the GPI between patients with tinnitus and controls with the

narrowband background noises of five different octave frequencies (0.25, 1, 2, 4, and 8 kHz) (93). However, in another ASR study with the 50-ms gap duration and 120-ms ISI, patients with tinnitus showed significantly less inhibition than controls with both high- and low-frequency background noises (30). Significant inhibitions of the N1-P2 complex with the 50-ms gap duration in the present study might be because the N1-P2 complex, which is generated by the auditory cortex, more closely reflects the cortical modulation than the ASR, which is generated by the subcortical areas.

At the 20-ms gap duration with 8 kHz-frequency background noise, a GPI deficit of the N1-P2 complex was observed in patients with tinnitus while controls displayed significant inhibition. Although there is no comparative human study using the ASR with the gap of 20-ms duration, most studies that have shown gap deficits of the ASR in tinnitus-induced animals had a cue onset timing of around 100 ms with 20- to 50-ms gaps inserted (32, 33, 107). In the present study, the 120-ms cue onset timing is the closest approximation of the timing condition used in the animal studies. Based on significant inhibitions of the N1-P2 complex at longer gap durations in patients with tinnitus, the gap may not be completely masked by tinnitus sound. Thus, a possible explanation for the inhibition deficit of tinnitus patient is that the relatively short cue onset timing and duration of 20-ms gap would allow less chance for higher cortical processing and could facilitate the effect of the partially filled gap in patients with tinnitus. Meanwhile, a previous psychoacoustic gap detection test reported that the threshold of average gap

detection in patients with tinnitus was below 10 ms with 25-dB SL tinnitus-pitch-matched background noise (31). (31). The PPI of the ASR is known to be modulated by high cognitive processes (56, 105, 108). Although the top-down modulation has complicated interactions with the bottom-up influences such as the ISI of the stimulus, the inhibition can be enhanced by attention in both humans and animals (109-111). The attention also increases the PPI of the cortical responses (112). Moreover, attention may have a significant effect on the GPI of the ASR in animals with noise-induced tinnitus (113). Because the psychoacoustic gap detection test requires the subject's attention, the effect of the partially filled gap could possibly be compensated by the attentive process in the psychoacoustic gap detection test. This hypothesis could be indirectly supported with the attentional enhancement of cortical responses to gaps in noise (77).

Second, the result at the 20-ms gap duration with 600 Hz-frequency background noise was assessed, since it is hypothesized that the gap inhibition deficit is expected only when the background noise is qualitatively similar to the tinnitus sound. In the present study, both groups showed the GPI deficit at the 20-ms gap duration with the tinnitus-pitch-non-matched frequency background noise. Thus, the hypothesis of tinnitus filling in the gap could not be confirmed in the present study, despite detection of the GPI deficit of patients with tinnitus with the tinnitus-pitch-matched frequency background noise. The deficits with low-frequency background noise might be due to the fundamentally different effects of background frequencies on gap processing,

as several studies have shown less inhibition even in controls at the relatively short gap durations with low-frequency background noise than with high-frequency background noise. Atcherson and colleagues (76) reported that the gap threshold in continuous narrowband noises to evoke the auditory N1 component increased with lower-frequency background noise. In their study, the clear N1 component was elicited by < 20-ms gap duration than with background noises of 1 or 4 kHz-frequency, but not 500 Hz-frequency. Consistent with these results, in some psychoacoustic gap detection studies, the requisite threshold of the gap duration increased with lower-frequency background noise (79, 80). Based on the Atcherson and colleagues' study (76), a frequency of ≥ 1 kHz is required to minimize the intrinsic effects of different background frequencies on gap processing to determine the tinnitus filling in the gap.

4.4.2. Consideration of Factors Affecting the N1-P2 Complex

Although high test-retest reliability of the sensory gating in the N1-P2 complex was reported (101), another study found reliable temporal stability only in P2, but not in N1 (114). The factors affecting stability of the N1-P2 complex, attentional modulation (112, 115) and the effect of drowsiness (116, 117) have been found. Because the attentive process could lead to lack of discrimination in the GPI paradigm, the passive listening protocol would be appropriate for human studies. In the present study, silent video clips were used to deviate participants' attention away from the auditory stimuli and

alleviate drowsiness because the passive listening protocol might increase drowsiness of some subjects. However, it is possible that some visual changes occurred within the inhibition timing (< 500 ms) before no-gap-intense auditory stimuli, which may lead to unexpected attenuation of the N1-P2 complex (118). Staring at a fixation point would be preferable to exclude the effect of the visual stimulus; however, it could increase drowsiness of subjects. Thus, decreasing testing gap conditions and trials and consequently, the required time of the experimental protocol is the optimal approach to prevent drowsiness. Gap durations that displayed discriminative responses (e.g., 50 ms in the ASR study and 20 ms in the present study) are recommended for future study. Moreover, the signal processing techniques that require less stimulus repetitions than conventional ensemble averaging could be applied. The narrowband noise showing more effective PPI than pure tone noises (119, 120) could be used to decrease the required time. Less number of trials also decreases the chance affected by short-term habituation, although the effect of short-term habituation was not observed in the present study. The counterbalanced order of testing conditions would be necessary to prevent the effect of long-term habituation based on the comparison result between the sequential and counterbalanced order in the present study. For other factors, nicotine (121) or caffeine (122), which are known to affect the PPI, should be controlled. Moreover, gender differences should be considered based on previous reports of less PPI in female than male individuals (103, 104). In the present study, gender differences of the GPI and amplitude were not observed

in both groups; however, the small sample size for each gender might have increased the standard errors. Separate comparison for each gender in controls and patients with tinnitus with sufficient sample sizes is required.

4.4.3. Limitations and Future Work

As the first comparative study using the GPI of the N1-P2 complex, subjects only who chose the 8 kHz tone as the most similar sound of tinnitus were recruited to exclude the potential effect of the background frequency. The tonal 8 kHz- patients with tinnitus were chosen as the subjects in the present study because the 8 kHz has been considered as one of most often reported tinnitus pitches (24). However, in the second tinnitus-matching test before the ALR measurement on the testing day, two patients could not confirm the most similar frequency with their tinnitus. This might be because the test frequency of the present study ended at 8 kHz frequency. This limitation occurred due to the limitation of output frequency of the testing device. The present study tried to exclude the possibility that actual tinnitus pitches of some patients were higher than 8 kHz by providing the "no similar sound" option in the tinnitus-matching test. Although the 8 kHz frequency is close to higher tinnitus pitches and could better hinder the recognition of the gap-prepulse than the 600 Hz frequency, the possibility of involvement of patients with a tinnitus frequency higher than the 8 kHz could not be excluded, which is a limitation of the present study. Thus, more precise tinnitus-pitch likeness rating methods (30, 123) including higher frequencies than 8 kHz would be

useful to hinder the gap recognition of patients with tinnitus and lead to detectable group difference. Moreover, stratification of patients with tinnitus by psychological distress level may provide useful information. In a previous study that compared two tinnitus subgroups (tinnitus complainers and non-complainers) with controls, the habituation deficit was found in the tinnitus complainers subgroup (88). Another study compared two tinnitus subgroups (high distress and low distress) with hearing loss-matched controls under the attended or unattended condition. In the unattended measurement condition, significantly diminished N1 amplitude was detected in patients with high tinnitus-related distress (124). The present study evaluated the N1-P2 amplitudes based on the findings of enhanced ASR amplitudes of patients with tinnitus in previous GPIAS studies. However, the patients with tinnitus displayed only weak tendency toward less amplitude, as compared to controls, and the difference was not significant. Patients with tinnitus who visited the hospital for treatment was recruited; therefore, the subjects of the present study were possibly of the tinnitus complainers' subgroup. Further correlation analysis on the amplitude and GPI of the N1-P2 complex with the quantified levels of stress or anxiety from tinnitus would be advantageous to detect group differences and better understand the heterogeneity of patients with tinnitus. Moreover, although only one channel ALR was recorded in the present study, the topographical analysis using multi-channel ALRs can provide more specific evidences such as responsible regions for the GPI deficit of the N1-P2 complex in patients with tinnitus. Simultaneous

measurement of the ASR and N1-P2 complex by attaching electrodes around eyes can provide information about the eyeblink synchronized with intense auditory stimuli. Comparative study of the two responses in the same controls and patients with tinnitus is required.

4.5. Conclusion

In conclusion, the effect of tinnitus on the N1-P2 complex in the GPI paradigm emerged only at the discriminative gap duration with the 8 kHz (tinnitus-pitch-matched) background frequency. Our findings suggested that the effect of tinnitus on the gap processing might be affected by the interactions between the bottom-up influences (stimulus properties) and top-down modulation (higher cognitive processing). The hypothesis of tinnitus filling in the gap could not be confirmed because both groups displayed the GPI deficits at the same gap duration with the 600 Hz (tinnitus-pitch-non-matched) background frequency. The background frequency of ≥1 kHz is suggested as the comparative background noise to minimize the intrinsic effects of different background frequencies on the gap processing.

Chapter 5. Thesis Summary and Future Work

5.1. Thesis Summary and Contributions

A high-fidelity AEP recording system with low noise and precise stimulus generation was developed, including the hardware and software. A single platform structure of this system can increase the operational stability and provide easy control of the system with users. The results of the performance evaluation indicated that this system can be used for the high-fidelity recording of the AEP in response to precise stimulus presentation. Real ABR and ALR recordings using the developed system were performed on human subjects. Typical morphologies of the ABR and ALR waveforms were confirmed and they were reproducible in all subjects. Flexible parameter setting and real-time raw data processing of this system will be beneficial for various clinical and research applications of the AEP signal.

The developed AEP recording system was actually applied in the human clinical studies to find the objective diagnostic evidence of tinnitus patients. Because the auditory system abnormality, especially in the auditory cortex, is considered as the main cause for chronic tinnitus, the ALR originated from the auditory cortex was adopted as the response index. By utilizing the flexibility in stimulus generation of the developed system, the GPI paradigm that has been used in animal studies was modified and applied in the context of human subjects. Because there have been no reports of sensory gating characteristics

by the gap-prepulse in humans, the ALR analysis with various stimulus conditions was performed in healthy normal-hearing subjects. First, significant inhibitions of typical ALR peaks in response to the gap-intense auditory stimuli were found. The N1-P2 complex best reflected the GPI in terms of suppression degree and test-retest reliability. It was also found that at least 100 stimuli repetitions were required to obtain a stable GPI ratio in one measurement. Finally, the gap length and ISI significantly affected the GPI ratios of the ALR. These findings could provide a normative data for the comparative study with tinnitus patients.

Next, the comparative study was performed with tinnitus patients and age- and hearing loss- matched controls. To my knowledge, no study has investigated the effect of tinnitus on the N1-P2 complex in the GPI paradigm. A discriminative gap duration showing the effect of tinnitus presence was found. It was interpreted that the appropriate cue timing and duration of the gap-pepulse would allow less chance involving high level cortical processing, which could compensate the effect of tinnitus, and then could show the group difference between tinnitus patients and controls. Thus, the N1-P2 complex of the ALR in the GPI paradigm may represent an objective measure of tinnitus.

In conclusion, the developed AEP recording system can be also applied to auditory studies of other neurodevelopmental disorders such as schizophrenia, depression, or autism. The flexibility of the developed AEP

recording system will be useful for new approaches using novel auditory stimulus paradigms. The findings of human clinical studies for objective tinnitus diagnosis can make a solid contribution as normative information.

5.2. Future Work

The AEP recording system can be improved by utilizing its flexibility in raw data processing. Many neuroscience researches try to control various factors affecting the experiment for the proof of hypothesis. Shortening the required test time can firmly contribute to minimize other effects, especially endogenous factors such as drowsiness or habituation. Obviously, the short test time can also decrease the chance involving external noises such as eye blink or motion artifact. Thus, the development of fast AEP extraction algorithms will be advantageous to conduct the clinical researches. Moreover, the real-time processing of the advanced algorithms on the system hardware will reduce the dependence on the external device. This approach will be also useful to decrease the amount of data communication. Moreover, the wireless AEP recording system can provide better convenience with users and subjects in clinics as well as in research laboratories.

The GPI study using the ALR should be continued toward the objective tinnitus diagnosis based on the findings of this thesis. The reproducibility should be confirmed in a considerable number of subjects. The effects of

different types of tinnitus and patients should be considered in the study design. Especially, the stratification of tinnitus patients by the psychological distress level may provide useful evidences to understand the heterogeneity of tinnitus. By exploiting the features found from clinical studies, physiological evidence-based classification algorithms can be applied for the automatic diagnosis.

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초 록

청성유발전위는 소리 자극 후 시간에 따라 청신경 시스템에서 발생하는 전위 반응이다. 청성유발전위는 객관적인 청력 검사 및 청신경 질환 진단 등의 목적으로 임상에서 활발하게 사용되고 있다. 또한, 다양한 신경발달질환들의 발생 기전 연구를 위한 측정 지표로도 활용되고 있다. 비침습적으로 측정되는 청성유발전위는 크기가 매우 작고, 소리 크기 및 주파수에 따라 민감하게 변화하기 때문에, 고품질의 청성유발전위를 측정하기 위해서는 우수한 저잡음 성능을 가지며 정확한 소리 제어가 가능한 측정 시스템이 필요하다. 또한, 고유한 소리 자극 구조와 청성유발전위 처리 기법을 이용한 임상 연구들이 최근 활발히 진행되고 있는데, 이를 위해서는 유연한 소리 자극 생성 및 청성유발전위 처리가 가능해야 한다.

따라서, 먼저 본 연구에서는 자유로운 소리 자극 제어 및 신호 처리가 가능한 고품질 청성유발전위 측정 시스템을 개발하고자 하였다. 또한, 별도의 전원 제어 및 소리 생성 장치들을 사용하지 않는 단일 기기의 형태로 개발하고자 하였다. 이를 위해 저잡음 아날로그 초단 회로부 및 어댑터 전원을 사용하기 위한 저잡음 전원 회로를 설계하였고, 이를 고해상도 아날로그 변환 및 우수한 출력 파워 성능을 갖는 소리 출력 회로와 통합하였다. 또한, 소리 자극 구조를 쉽게 설정할 수 있으며 병렬 데이터 처리 구조를 통해

실시간 청성유발전위 처리가 가능한 소프트웨어를 개발하였다. 개발된 청성유발전위 측정 시스템은 평균 0.6 μV_{RMS} 이하의 우수한 시스템 잡음 수준을 보였으며, 소리 출력에 있어 미국 표준협회에서 국제 표준으로 제시하는 출력 크기 (±1.5 dB SPL 이하) 및 주파수 (±1% 이하) 정확성 기준들을 모두 만족하였다. 또한, 실제 청성뇌간반응 및 청성후기반응이 재현성있게 측정 가능함을 확인하였다.

둘째로, 개발된 청성유발전위 측정 시스템의 자유로운 소리 자극 제어 및 신호 처리 기능을 활용하여 실제 인간 대상 임상 연구를 수행하고자 하였다. 주요 이과 질환들 중의 하나인 이명 분야에 있어, 객관적인 진단법의 부재는 대표적인 미충족 임상 수요로 제시되고 있다. 따라서, 동물 이명 연구에서 사용되어 온 고유한 소리 자극 구조인 펄스전 간격법의 측정 지표를 청성후기반응으로 변형하여 인간 대상으로 객관적인 이명 진단법을 도출하기 위한 임상 연구를 수행하였다. 먼저 청성후기반응 중 N1−P2 크기가 펄스전 무음 간격에 의한 억제율 및 재현성 측면에서 가장 우수한 지표임을 확인할 수 있었다. 또한, 단일 측정에서 무음 간격에 의한 억제율이 안정화되는 최소 반복 횟수를 발견하였고, 이를 통해 향후 연구에서의 측정 소요 시간을 단축할 수 있었다. 마지막으로, 무음 간격 및 배경 주파수를 변경하면서 다양한 조건의 펄스전 간격법 소리 자극들을 이명 환자 및 대조군에

인가하는 비교 임상 연구를 수행하였다. 이를 통해, 청성후기반응에서 이명의 효과 (p = .001)가 나타나는 펄스전 간격법의 소리 자극 조건을 발견하였다. 따라서, 본 연구에서 제안하는 펄스전 간격법의 반응 지표로 청성후기반응을 이용하는 패러다임은 객관적인 이명 진단의 도구로써 가능성이 있음을 확인하였다.

주요어: 청성유발전위, 저잡음 생체신호 측정회로, 객관적 이명 검사, 펄스전 간격법, N1−P2 크기
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