# Change in Acoustic Startle as an Indicator of Continuous Tonal Tinnitus

by

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Abstract: Currently, there is no accepted objective measure of tinnitus in humans. The gap prepulse inhibition of acoustic startle (GPIAS) paradigm is an objective measure that has been used in the animal model to identify tinnitus based on the theory of tinnitus filling in the silent gap that would normally promote startle inhibition. The current study applied the GPIAS paradigm in human subjects with normal hearing thresholds without hyperacusis. Individuals with continuous tonal tinnitus (N=31) characterized their tinnitus by adjusting a signal to match the frequency, bandwidth, and intensity. These individual parameters were used to create maximally matched background sounds in the GPIAS paradigm for each subject. A group without tinnitus (N=8) also participated using the averaged parameter values of the background sound from the group with tinnitus. Startle inhibition percentage was calculated by comparing ocular EMG blinking amplitudes between gap embedded conditions and the condition without a gap. As expected, the group with no tinnitus revealed startle inhibition as evidenced by reduced EMG blink amplitudes when the background sound was interrupted by a silent gap prior to the startle impulse (100 dB SPL white noise). The group with tinnitus did not have a significant startle inhibition in this same condition supporting the theory that the background sound carefully matched to their tinnitus eliminated the perception of a silent gap, thereby removing the cue that would produce startle inhibition. Gradually increasing the contrast between the individual's continuous tonal tinnitus and ongoing background sound leads to a nonlinear change in startle inhibition percentage, providing guidelines for how closely the background sound needs to match the tinnitus of an individual in

order to get the expected result of no startle inhibition when tinnitus is filling in the gap. Collectively, these findings support the use of the GPIAS paradigm for objectively identifying continuous tonal tinnitus in humans. Further, certain deviations in frequency, intensity, or bandwidth in the ongoing background sound from the tinnitus match result in startle inhibition, which may help explain the inconsistent findings across human GPIAS studies and allow more confidence for animal researchers to use GPIAS for animal tinnitus studies.

## Table of Contents

## List of Tables

## List of Figures

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## 1.0 Abbreviations

AC: auditory cortex

ANF: auditory nerve fibers

CAP: compound action potential

DCN: dorsal cochlear nuclei

EEG: electroencephalography

EMG: electromyography

EPSP: excitatory postsynaptic potentials

GABA: gamma-Aminobutyric acid

GIN: gaps in noise

Gly: glycine

Glu: glutamate

GPIAS: gap-prepulse inhibition of the acoustic startle

HCN: hyperpolarization-activated cyclic nucleotide

IC: inferior colliculi

IHC: inner hair cell

IPSP: post-synaptic potential

KV: voltage-gated potassium channel

LTD: long-term depression

LTP: long-term potentiation

MGB: medial geniculate body

MMN: mismatch negativity

NMDA-R: N-methyl-D-aspartate receptor

OHC: outer hair cell

SPIAS: sound-prepulse inhibition of acoustic startle

SOC: superior olivary complex

STDP: spike-timing-dependent plasticity

SFR: spontaneous firing rate

SPL: sound pressure level

VCN: ventral cochlear nuclei

DL: difference limen.

## 2.0 Introduction

Tinnitus is the perception of sound without an existent outside sound source. Tinnitus affects people both with and without hearing loss, and may significantly impact an individual's quality of life (Cartocci et al., 2012). Tinnitus creates a societal cost burden of more than $15 billion per year in the US that is shouldered by individuals, families, the health care system, and the workplace (Anders et al., 2010; Nondahl et al., 2011). A retrospective study by (Goldstein et al., 2015)estimated the healthcare cost of tinnitus to be around $660 per patient per year (which is about $21 billion per year) in the United States based on the 2013 US Census and an estimated 10% prevalence of tinnitus in the adult population. Earlier survey results revealed over 35% of the U.S. population experienced tinnitus (Gentile, Schein, & Haase, 1967). At the same time, tinnitus is a financial burden for other countries in the world. For example, in the Netherlands, Maes et al. (2013) estimated the mean annual tinnitus-related healthcare cost per patient to be around $1,700.

Additionally, tinnitus is the most prevalent military service-connected disability, with more than 1 million Veterans receiving disability compensation for hearing loss and tinnitus (Veterans Administratsion, 2015). Tinnitus impacts the pediatric population, as well. According to a survey in 2013, about 1.6 million American adolescents report having tinnitus, a 4.7% prevalence rate of chronic tinnitus in the adolescent population in the United States (Mahboubi, Oliaei, Kiumehr, Dwabe, & Djalilian, 2013). Although there are programs available to help people manage and cope with their tinnitus, currently there is no cure. To target any type of treatment, one would need to identify the generation site or sites of tinnitus. The fact that there is no objective test in humans to

verify the presence of tinnitus makes exploring the underlying mechanism(s) of tinnitus a problematic task.

Researchers have turned to an animal model to try to identify the possible sites of tinnitus generation in the peripheral and central auditory system. Although known causes of tinnitus (e.g., ototoxic drugs and loud noise) can be applied to an animal, the challenge remains that the animal cannot report if they perceive tinnitus. This challenge has led researchers to develop psychoacoustic and behavioral test paradigms that might verify that an animal is experiencing tinnitus. All these techniques, however, cannot be validated in the animal, and therefore, the resulting data related to tinnitus is continually called into question. If one could verify the techniques applied in animal research in humans, one might be able to capitalize on the animal data to move ahead toward possible, targeted interventions.

## 3.0 Identifying the Generation Sites of Tinnitus

The generation site of tinnitus has been explored for years in animal studies, with loci within the auditory system being the most common focus of these investigations. The dorsal cochlear nuclei (DCN) has been identified as one site responsible for tinnitus generation but not for the persistence of tinnitus (Brozoski & Bauer, 2005; Brozoski, Wisner, Sybert, & Bauer, 2012). There is no broad consensus as to the other loci responsible for tinnitus generation. One can grossly classify tinnitus based on its peripheral and central loci where there is a change in neural activity after the damage is applied to the animal model (e.g., ototoxicity, noise) potentially creating tinnitus. Some researchers report that tinnitus has a peripheral generation (Cazals, Horner, & Huang, 1998; Guitton et al., 2003). For example, animal studies found that altered spontaneous eighth nerve activity reflects the presence of salicylate-induced high-pitch tinnitus (Cazals et al., 1998), and hypothesize that salicylate induces tinnitus through activation of cochlear N-methyl-D-aspartate receptors (NMDA-R) (Guitton et al., 2003). While others have proposed a central generation theory, which is beyond the auditory nerve, because some studies have found that ablation of the cochlea does not result in a spontaneous firing rate (SFR) change at the dorsal cochlear nuclei (DCN), and the sectioning of the auditory nerve does not always abolish tinnitus (Bledsoe et al., 2009; House & Brackmann, 1981; Kaltenbach, Zhang, & Finlayson, 2005; Noreña & Eggermont, 2003; Zacharek, Kaltenbach, Mathog, & Zhang, 2002). Thus, tinnitus seems able to originate peripherally, centrally, or both, which likely varies depending on the etiology.

## 4.0 Sensory Deafferentation and Non-Deafferentation Triggers of Tinnitus Induction

Like the substantial uncertainty of tinnitus study findings related to generation loci, multiple putative neurophysiological mechanisms for tinnitus generation cannot be identified consistently across the auditory system. The most commonly reported effects of tinnitus-inducing agents on neurons in the auditory system are changes of (1) **spontaneous burst-firing activity** and (2) **neural synchrony**, both of which further build upon the concept of **central gain enhancement**. All three concepts belong to the **deafferentation** triggers of tinnitus generation**.** While deafferentation can explain some of the tinnitus-related neural phenomena, a **non-deafferentation** mechanism must be included to explain the other tinnitus associated neural activities. **Excitotoxicity** and **activity-dependent plasticity** are consistent with the concept of non-deafferentation (Figure 1).

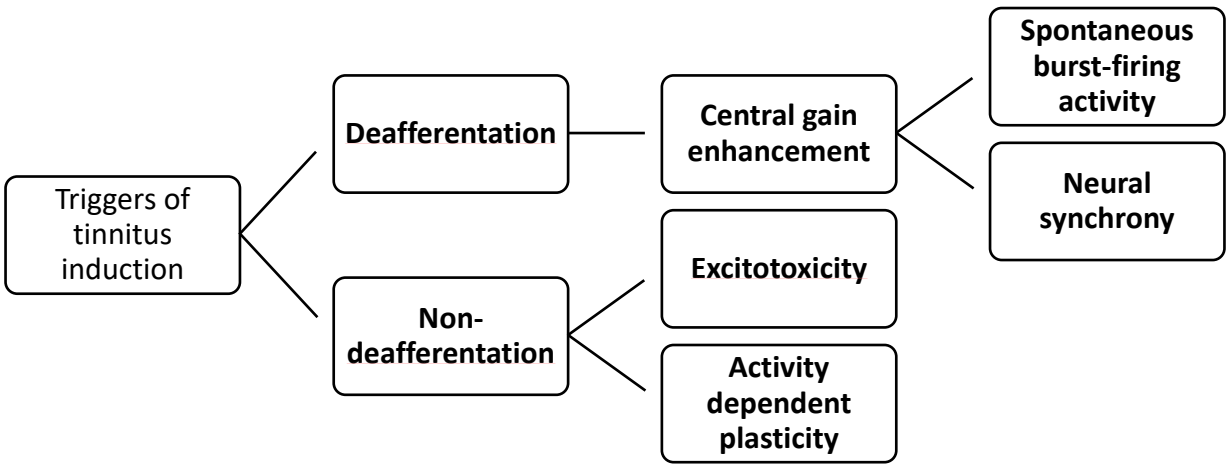



**Figure 1.Hierarchical structure of triggers of tinnitus induction**

## 4.1 Deafferentation

Sensory deafferentation is a loss or significant reduction of the sensory input caused by interruption of sensory fiber connections. Noise-induced sensory deafferentation could be a result of hearing loss and is often accompanied by reorganization of the tonotopic map in the thalamus and auditory cortex of cats (Kamke, Brown, & Irvine, 2003; Norena, 2005). Along with the remapping process found in animals, a human study has reported an edge effect, which means an original characteristic frequency of neurons corresponding to the deafferentated region shifts towards the edge frequency of hearing loss. If reflected in a frequency discrimination test, an improvement of test performance at the edge frequency of hearing loss should be observed (Moore & Vinay, 2009).

Sensory deafferentation leads to spontaneous firing rates (SFR) and neural synchrony changes varying across timeline and locations. Therefore, tinnitus is often viewed as a deafferentation disorder triggered by the loss of normal input from the auditory periphery. Evidence for a deafferentation mechanism of tinnitus comes from a wide range of clinical and experimental observations. Firstly, tinnitus is most commonly associated with hearing loss. Secondly, tinnitus also can be induced by surgical damage as well as compression of the eighth nerve. These conditions involve impairment of peripheral auditory functions, so there is a good reason for human observations alone to suspect that loss of peripheral function and peripheral input is critical triggers of tinnitus.

Animal models have yielded evidence consistent with a deafferentation induced mechanism of tinnitus. In DCN, the induction of tinnitus-related hyperactivity is correlated with the loss of outer hair cells. Since the granule cell domain of DCN receives input from outer hair

cell originated type II spiral ganglion neurons, it has been hypothesized that loss of outer hair cells may induce hyperactivity in the DCN by causing damage of peripheral input to the granule cell system (Figure 2). Moreover, activation of granule cells influences the level of activity of the fusiform cells (i.e., principal cells) of the DCN, the likely generators of tinnitus signals.

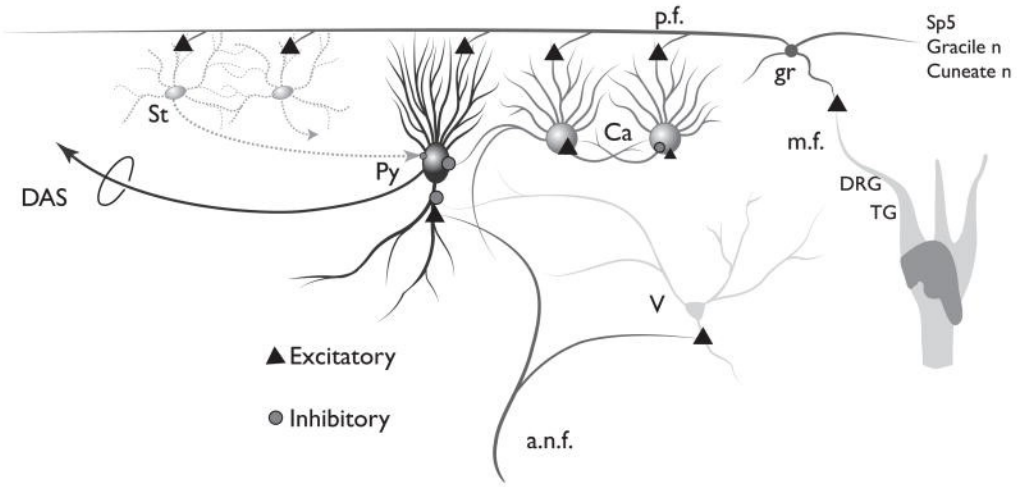



**Figure 2. Schematic of DCN circuitry (with permission from Shore 2007). Pyramidal cells (Py) in layer II of the DCN receive inputs on their basal dendrites from auditory nerve fibers (a.n.f.) and vertical (v) cells. The apical dendrites of the pyramidal cells receive inputs from the parallel fiber axons (pf) from granule cells (gr) in the VCN. In contrast, their cell bodies receive inputs from cartwheel (Ca) and superficial stellate (st) cells. Projections from the trigeminal ganglion (TG), spinal trigeminal nucleus (Sp5), dorsal column nuclei (Gracile and cuneate n), and the dorsal root ganglion (DRG), synapse on granule cells.**

Deafferentation also can involve loss of input to auditory structures from non-auditory areas, such as the somatic system. These non-auditory inputs can be merged into the auditory pathway at multiple levels. For instance, the output of DCN is adjusted by the granule cell system, which receives input from the auditory system, somatosensory system, along with multiple other pathways. Since activation of the granule cell system is known to affect the level of spontaneous

activity, conditions in which inputs from these areas are impaired or damaged could affect the output of the dorsal cochlear nucleus via their effects on the granule cell system.

There are two general mechanisms behind the deafferentation, which induce tinnitus-related activity in the central auditory system by activating neural plasticity. The most frequently hypothesized mechanism is a shift from balanced excitation/inhibition inputs between synapses towards the excitatory side. Such a shift could involve direct loss of inhibitory inputs (disinhibition), an increase in excitatory inputs, or both, and thus could result in increased central gain (Turrigiano, 1999). Several lines of evidence showed that both a loss of inhibition and an increase in excitation occur centrally after the loss of auditory nerve input and that such changes involve plasticity (Kim, Morest, & Bohne, 1997; Milbrandt, Holder, Wilson, Salvi, & Caspary, 2000; Wang et al., 2009; Whiting, Moiseff, & Rubio, 2009). A less mentioned second mechanism is an increase of neuron excitability attributed to the inherent membrane adjustments. This mechanism has been established based on noise-exposed animal models. Such membrane adjustment involves up or down regulations of specific ionic conductance channels in DCN (Holt et al., 2006; Li, Choi, & Tzounopoulos, 2013; von Hehn, 2004) or increases in membrane resistances in the ventral cochlear nuclei (VCN) (Francis & Manis, 2000).

### 4.1.1 Central Gain Enhancement

Central gain is the paradoxically enhanced neural activity in central auditory structures at suprathreshold intensity levels, as a result of loss or reduction of sensory deafferentation.

Why does central gain enhancement occur? One hypothesis is that central gain enhancement maintains a mean neural activity at a set value and preserves neural coding efficiency

when the central auditory system faces sensory deafferentation. A concept that has been forwarded by some researchers is that tinnitus can be a result of the misbalance between central gain and peripheral SFR. Norena et al. (2005) gave the example that if sensory deprivation happens, with intact peripheral SFR, the central gain will then increase and eventually results in tinnitus. However, in situations where sensory deprivation happens but with a significant reduction of peripheral SFR, then the increase in central gain is counterbalanced by a more considerable peripheral reduction of SFR; thus, no tinnitus would occur.

A study from Salvi et al. hypothesizes that central gain starts at the IC (see Figure 3 & Figure 4). Multiple variables can contribute to the temporal profile of gain enhancement, including (1) a decrease in inhibitory synaptic responses, (2) an increase in excitatory synaptic responses, or (3) alterations to intrinsic neuronal excitability.

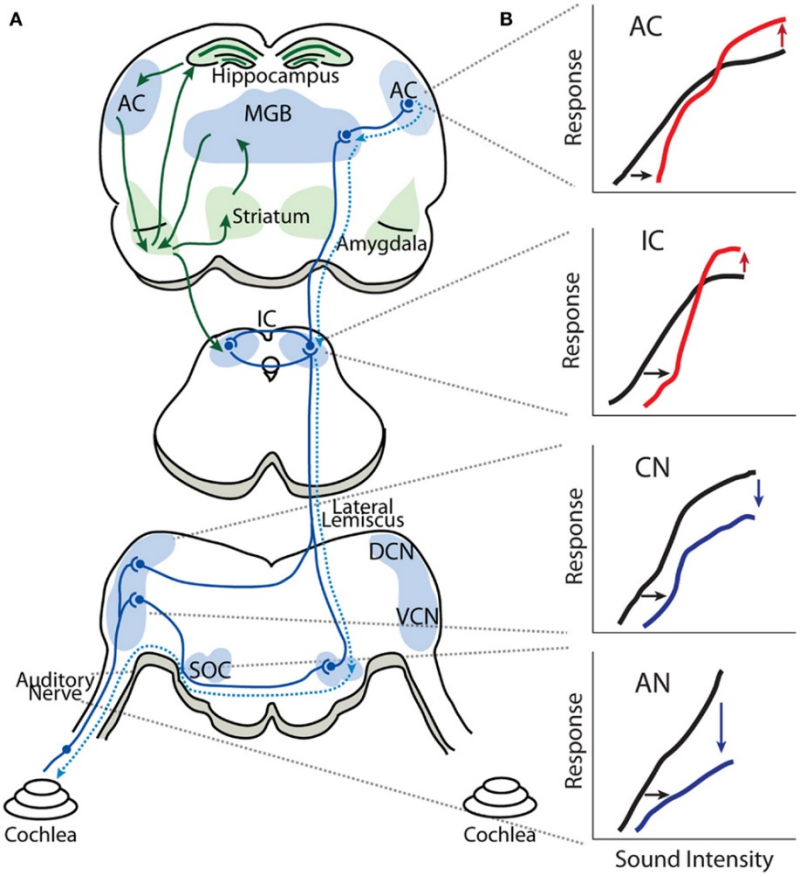



**Figure 3.Gain enhancement in the central auditory system. (with permission from Auerbach, 2014)**

**Schematic showing the general anatomical organization of the auditory system. The nuclei and areas of the auditory system are highlighted in blue. The ascending anatomical projections are depicted with solid blue lines, whereas the dotted blue lines represent descending projections. Limbic regions that respond to auditory stimuli and display some evidence of central gain enhancement are highlighted in green. Schematics of intensity-level functions collected from the auditory nerve (AN), cochlear nucleus (CN), inferior colliculus (IC), and auditory cortex (AC). The black lines represent baseline intensity-level functions. Cochlear damage via noise or ototoxic drug exposure results in depression of sound-evoked responses in lower auditory structures (blue lines). Still, it results in enhancement of suprathreshold responses in higher areas (red lines), despite thresholds being shifted (black arrows). SOC, superior olivary complex; VCN, ventral cochlear nucleus; DCN, dorsal cochlear nucleus; IC, inferior colliculus; MGB, the medial geniculate body; AC, the auditory cortex.**

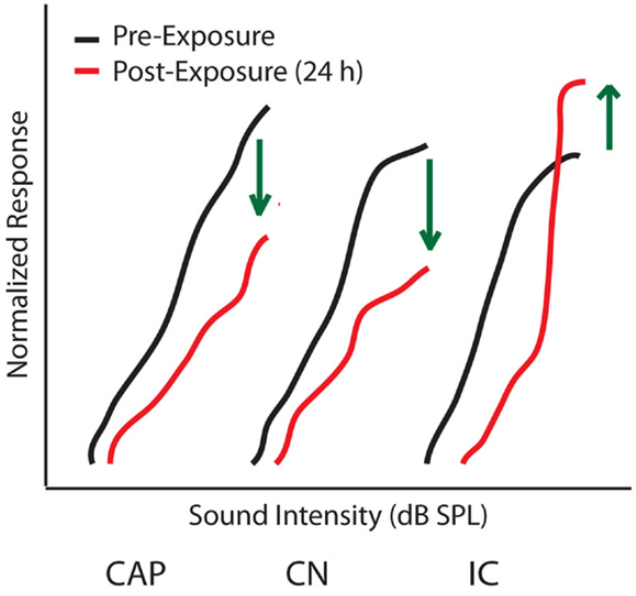



**Figure 4.Origins of central gain enhancement (with permission from Auerbach, 2014). Schematized data for amplitude-level functions to a 1 kHz tone chronically recorded from chinchillas at the round window. Compound action potential (CAP), cochlear nucleus (CN), and inferior colliculus (IC), before (black lines) and 24 h after (red lines) noise-exposure of 105 dB SPL at 2.8 kHz for two h. Green arrows indicate the direction of amplitude change after noise-exposure. Responses are normalized to the maximum response before the noise-exposure**

#### 4.1.1.1 Spontaneous Burst-Firing Activity

Noise exposure and salicylate-like ototoxic drugs, which are known to induce tinnitus, often cause changes in a specific type of activity called bursting discharges, also called spontaneous burst-firing or spontaneous firing (SF), in the auditory system. Chronic increases in bursting activity have been observed in the auditory nerve as well as the DCN after noise exposure, and found in the IC following salicylate and noise exposure; thus tinnitus may be induced by an increase in spontaneous firing rate (SFR) (Brozoski, Bauer, & Caspary, 2002; Kaltenbach &

Afman, 2000; Kaltenbach, Zhang, & Afman, 2000; Mulders & Robertson, 2009). Periodicities in burst-firing are crucial to the ability of neurons to encode the frequency of sounds. If SFR is increased, then periodicities in a restricted frequency range also might be increased, and this could lead to the perception of a tinnitus-like sound in a correspondingly restricted pitch range. At the same time, if SFR is decreased or remains unchanged, then tinnitus would not be generated. Following this rule, tinnitus cannot be solely explained by SFR increase. SFR has been explored throughout the auditory system of animals with suspected tinnitus after noise or drug exposure, and findings indicate that SFR can increase, decrease, or remain unchanged at each level of the auditory system. There is no clear explanation of non-changed SFR. The decrease of SFR can be a consequence of inner hair cell (IHC) stereocilia damage (Liberman & Dodds, 1984). That is to say, SFR is related to the spontaneous release of synaptic vesicles from IHCs. The rate of release of these vesicles depends on the resting membrane potential of IHCs, which is determined by the rate of resting current flow across the apical surface of the cell (through the stereocilia membrane). As a result, the spontaneous transmitter release is decreased, and so is the SFR of auditory nerve fibers.

Additionally, SFR change directions are associated with factors including etiology of tinnitus (i.e., noise trauma or ototoxic drugs), drug type and dosage, drug delivery method (i.e., oral or intravenous administration), and timing discrepancy (i.e., SFR may vary across timeline) (Eggermont & Kenmochi, 1998; Jastreboff, Brennan, & Sasaki, 1991; Kaltenbach et al., 1998; Norena, 2003; Ralli et al., 2010). Noise type, exposure intensity, and duration of exposure can change the results of SFR as well. Table 1 provides a summary of the effects of drugs and noise on SFR in tinnitus. This table describes the factors mentioned above, except for drug dosage, noise

type, noise exposure intensity, and noise exposure duration. These factors are not always reported in studies.

**Table 1. Effects of drugs and noise on spontaneous firing rate change in tinnitus (↑ indicates a significant increase; ↓ indicates a significant decrease; ≈ indicates no change; NS, not studied.)**

| | The direction of SFR Changes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Salicylate | | Quinine | | Cisplatin | | Noise trauma | |
| ANF | ≈ cat | (Stypulkowski, 1990) | ↓ guinea pigs | (Mulheran, 1999) | NS | | ↓ cat | ( Liberman & Kiang, 1978) |
| | ≈ gerbil | (Mueller, Oestreicher, Arnold, & Klinke, 2000) | | | | | | |
| | ↑ cat | ( Liberman & Kiang, 1978) | | | | | | |
| DCN | ↓ hamster | (Finlayson & Kaltenbach, 2009) | NS | | ↑ rats | (Kaltenbach et al., 2000) | ↑ hamster | (Kaltenbach et al., 1998) |
| | ≈ rats | (Chang, Chen, Kaltenbach, Zhang, & Godfrey, 2002) | | | | | | |
| IC | ↑ mice | (Ma, Hidaka, & May, 2006) | NS | | NS | | NS | |
| | ↑ chinchillas | (Bauer, Turner, Caspary, Myers, & | | | | | | |

**Table 1 (continued)**

| | The direction of SFR Changes | | | | | | |
|---|---|---|---|---|---|---|---|
| | Salicylate | | Quinine | | Cisplatin | Noise trauma | |
| AI | ≈ cats | (Ochi & Eggermont, 1996) | ≈ cats | (Ochi & Eggermont, 1997) | NS | ↑ cats | (Norena, 2003) |
| | ↑ rats | (Sun et al., 2009) | | | | | |
| | ↓ rats | (Yang et al., 2007) | | | | | |
| AII | ↑ cats | (Eggermont & Kenmochi, 1998) | ↑ cats | (Eggermont & Kenmochi, 1998) | NS | NS | |

In summary, only SFR increase theoretically contributes to tinnitus, while unchanged or decreased SFR does not. However, not only increased SFR but also unchanged or decreased SFR has been observed throughout the auditory system in several types of animals that are exposed to noise or drugs, which are expected to result in tinnitus. On the one hand, this variation in SFR change might be explained by the IHC status along with several other factors. On the other hand, Norena and Farley (2013) suggested that spontaneous activity might be the by-product of another network-level change that occurs earlier (Figure 5), instead of being a generalized mechanism of tinnitus per se (Amaral & Langers, 2015; Noreña & Farley, 2013). The hierarchically earlier change is spike-timing-dependent plasticity (STDP), which is under the category of the non-deafferentation triggers of tinnitus and will be discussed in a following section of this manuscript.

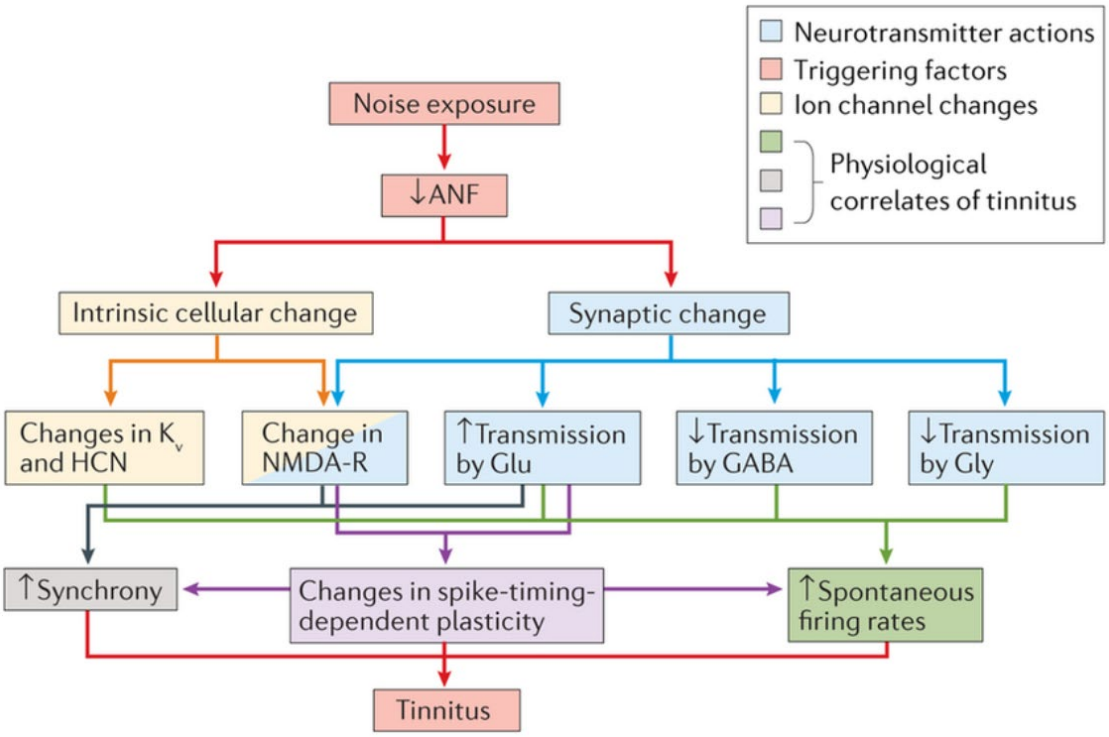



**Figure 5.Mechanisms that contribute to increased STDP, SFR and synchrony in the DCN (with permission from Shore, Roberts, & Langguth, 2016). STDP: spike-timing-dependent plasticity, SFR: spontaneous firing rate, ANF: auditory nerve Fibers, HCN: a hyperpolarization-activated cyclic nucleotide, NMDA-R: N-methyl-D-aspartate receptor, Glu: glutamate; GABA: gamma-Aminobutyric acid, Gly: glycine, $K_V$: voltage-gated potassium channel**

### 4.1.1.2 Neural Synchrony

Some researchers have tried to explain the generation of tinnitus through a theory of micro-change in the temporal pattern of spontaneous discharges since enhanced synchrony of inputs is another way to increase the probability of making a target cell fire. If the inter-spike interval within the burst is shorter than the time constant for the integration of excitatory inputs of a target cell, the excitatory post-synaptic potentials (EPSP) will summate, thereby increasing the probability of

firing in the post-synaptic cell. Therefore, the enhanced synchrony (i.e., temporal coherence) between discharges at the auditory nerve or cortical levels has been promoted as a cause of tinnitus.

In addition to this assumption, there is evidence for an increase in the synchrony of discharges among neurons in the IC following noise exposure and the auditory nerve fibers following salicylate treatment in animals. It is suggested by increases in the amplitude of 200 Hz and 900 Hz peaks in the frequency spectrum of ongoing ensemble activity. It means that instead of impulses being randomly related across the neural population, the impulses become increasingly coincident. Moreover, neurons showing increased synchrony occur in frequency bands of the hearing loss that are also the areas in which tonotopic map reorganization occurs in cats (Rajan & Irvine, 1998). Multiple animal studies found enhanced neural synchrony in noise exposure, which is expected to cause tinnitus (Noreña & Eggermont, 2003; Wu, Martel, & Shore, 2016).

Human studies also found evidence of neural synchrony increase in tinnitus. For example, neural synchrony is represented by high-frequency oscillatory dynamics in tinnitus that occur within distinct episodes of magnetic slow-wave (~4 Hz) activity in auditory regions, which is enhanced in tinnitus (Weisz, Wienbruch, Dohrmann, & Elbert, 2005). Weisz et al. (2007) believed that deafferentation prompts permanent alterations in the ongoing oscillatory dynamics in the central auditory system. This alteration results in enhanced slow-wave activity (~4 Hz) facilitating gamma activity (25~100 Hz) as a neural code of tinnitus (Weisz et al., 2007).

#### 4.1.1.3 Tinnitus and Hearing Loss

An intriguing question is why tinnitus does not always accompany hearing loss? If tinnitus is the result of deafferentation triggered increase in SFR, synchrony, or both, and possibly

involving non-auditory inputs to these centers, then why do many people with deafferentation including hearing loss, have no tinnitus? The conceivable explanation is that the direction of the shift in the balance of excitation and inhibition following cochlear injury may depend on the pattern of cochlear injury. Tinnitus induction would be expected to occur when there is more degeneration centrally of inhibitory than excitatory neurons, causing disinhibition and an increase in excitation. However, specific patterns of peripheral injury may not be enough to shift the balance of excitation and inhibition or could even favor a shift toward the side of greater inhibition. This assumption is supported by the fact that when a cochlear injury is induced by cisplatin and is restricted to outer hair cells, there is a strong relationship between the degree of centrally recorded hyperactivity and the amount of outer hair cell loss. Still, when the outer hair cell loss is accompanied by the disarray of stereocilia, activity is not elevated centrally. When the inner hair cell injury becomes more severe, or outer hair cell loss is accompanied by inner hair cell loss, hyperactivity is observed. This phenomenon suggests that the effect of peripheral injury on central auditory activity depends on the balance and type of injury to the two hair cell populations and their connecting primary afferents.

### 4.2 Non-deafferentation

Deafferentation is not the only triggering mechanism by which tinnitus-related activity could be induced. Some inducers of tinnitus may act through non-deafferentation mechanisms, such as **excitotoxicity** or **activity-dependent plasticity**.

### 4.2.1 Excitotoxicity

Excess release of excitotoxic neurotransmitters in the brain caused by acoustic overstimulation could lead to degeneration of second-order neurons (i.e., the neurons connecting the spinal cord or brainstem to thalamus). Glutamate is the most common excitatory and most powerfully excitotoxic neurotransmitter in the nervous system. It is also the excitatory transmitter of hair cells, auditory nerve fibers, granule cells of the cochlear nucleus, and the principal projection neurons that make up the ascending auditory pathway. Typically, the toxicity of this transmitter is prevented by its reuptake following its release by the presynaptic membranes. However, under certain conditions, such as when there is excessive sound stimulation, glutamate is released in excess, and this excess can sometimes crush the reuptake mechanism and lead to glutamate accumulation in the synaptic cleft. The resultant collection of glutamate in the synaptic cleft would thereby trigger excitotoxicity. Indirect evidence in support of this theory is that the degeneration of second-order neurons occurs in broader areas of the cochlear nucleus well beyond zones of peripheral deafferentation, which can only be alternatively explained by the excitotoxic injury in the central auditory system. This loss of second-order neurons would shift the balance towards excitation in the central auditory system and eventually induce tinnitus.

### 4.2.2 Activity-Dependent Plasticity

The commonly described mechanism by which synaptic excitability and spontaneous activity level of neurons are chronically shifted is long-term potentiation (LTP). LTP is a long-term enhancement in synaptic transmission between two synchronously stimulated neurons. LTP

leads to a sensitization of neurons to their inputs, which is demonstrated as an amplified response of the post-synaptic neuron as a reaction to its excitatory inputs (Shouval, 2010). LTP also is associated with an increase in spontaneous activity (Fernández-Ruiz, Makarov, & Herreras, 2012). Thus, if LTP occurs in the auditory system, it seems likely that the affected neurons would become hypersensitive and spontaneously hyperactive. The opposite process of LTP is long-term depression (LTD), which is displayed as a reduced response of neurons as a reaction to its inhibitory inputs. LTP and LTD have been implicated as neural mechanisms of learning memory, and are known to be ubiquitous phenomenon throughout the brain (Cobar, Yuan, & Tashiro, 2017; Wang, Wang, & Scheich, 1996).

Now we know what the demonstrations of LTP/LTD are and what their possible roles are in tinnitus generation, but what causes LTP specifically in auditory neurons? LTP can be induced in various auditory centers by synchronous stimulation of presynaptic and post-synaptic neurons, which is like what occurs to LTP in other sensory systems. The changes of LTP and LTD have been demonstrated in tinnitus animal models in the DCN, IC, and auditory cortex. Tinnitus inducers such as noise and ototoxic drugs might change the spike-timing-dependent plasticity (STDP) and thereby cause induction of LTP Such an STDP rule has been found across DCN, IC, and AC.

#### 4.2.2.1 Spike Timing Dependent Plasticity (STDP) Theory in Tinnitus

Plasticity between synaptic connections is a fundamental characteristic of learning processes. Long-term potentiation (LTP) or long-term depression (LTD) can enhance or diminish synaptic connections, respectively. The classical STDP is bidirectional and order-dependent (Figure 6). LTP happens when presynaptic spikes lead post-synaptic spikes by up to 20ms (pre-

to-post-paring), and LTD happens when post-synaptic spikes lead presynaptic spikes and EPSPs by up to 20~100ms (post-to-pre-paring) (Dan, 2006; Markram, Gerstner, & Sjöström, 2011). Strengthening or weakening the synapses is dependent on effectiveness, which is to say that ineffective synapses will be reduced while the active synapses get strengthened (Abbott & Nelson, 2000).

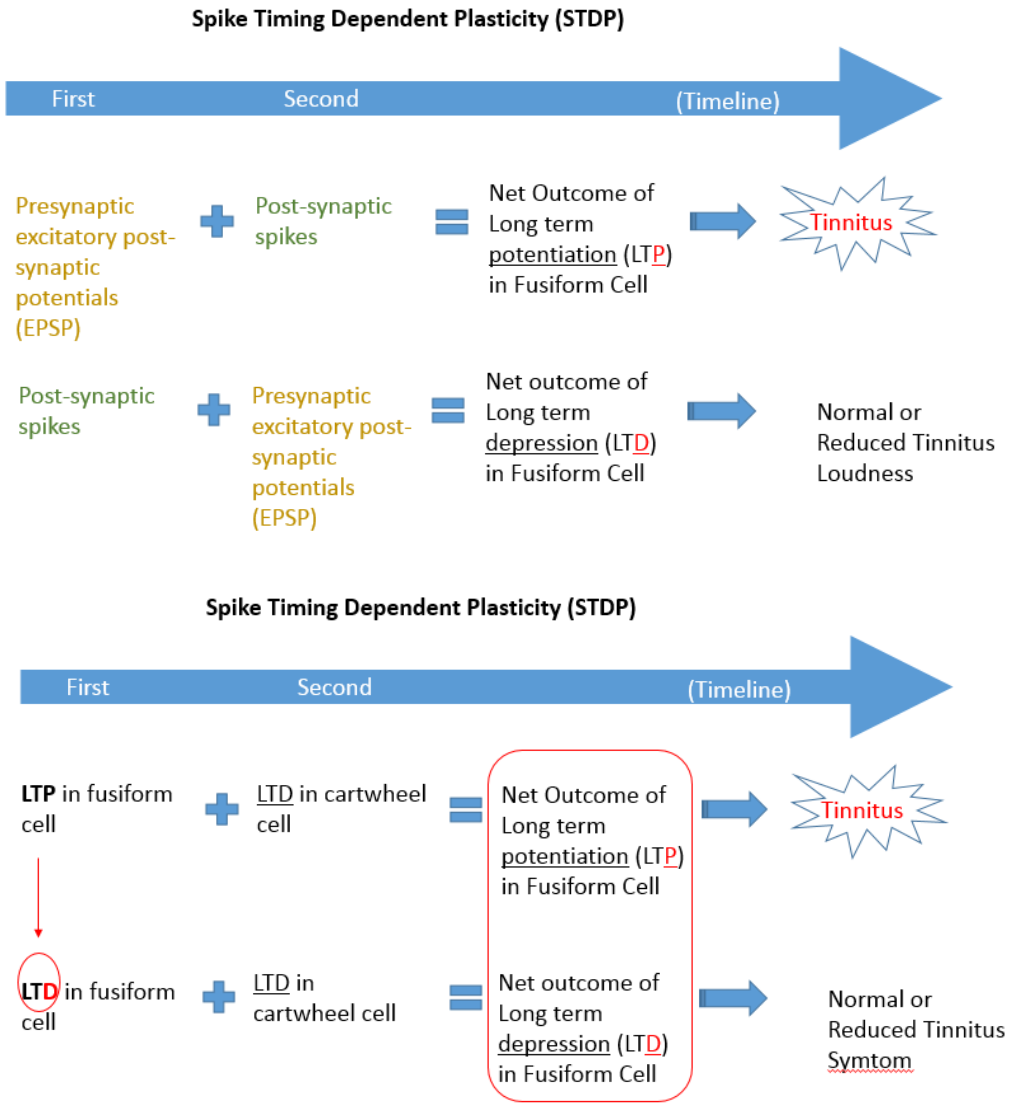



**Figure 6.Simplified schematic of STDP in DCN (based on findings from Tzounopoulos 2004 and Koehler & Shore 2013)**

Plasticity depends on 60~100 times of pre-post spike paring. This phenomenon is called Hebbian STDP since it strengthens synaptic inputs that lead and contribute to post-synaptic firing, and at the same time depresses inputs that are uncorrelated with post-synaptic spikes. If the Hebbian STDP learning rule is violated, the process is called as Anti-Hebbian STDP. Thus, the

order of spike timing is the first generation of features in STDP that are discovered and deemed to be the critical factor that controls plasticity (Figure 7).

Later on, firing rate and depolarization are recognized as other factors in synaptic plasticity (Sjöström, Turrigiano, & Nelson, 2001). The relevance of spike timing varies across synapses with strong spike-timing dependence, which is classical STDP, being restricted to specific dendritic zones and activity regimes. Four primary forms of STDP (Figure 7) include (1) Hebbian STDP that is equally balanced between LTP and LTD, (2) LTD-biased Hebbian STDP, (3) Anti-Hebbian STDP that has both LTP and LTD and (4)Anti-Hebbian STDP that has only LTD (Celikel, Szostak, & Feldman, 2004; Fino, Deniau, & Venance, 2008; Froemke, Poo, & Dan, 2005; Letzkus, Kampa, & Stuart, 2006; Safo & Regehr, 2008). Of note, the most common anti-Hebbian STDP only has LTD.

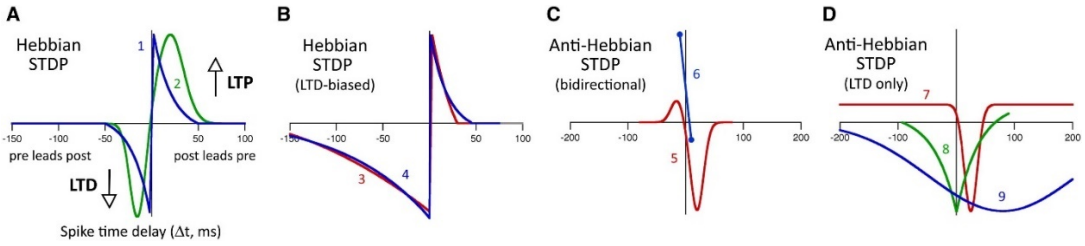



**Figure 7.Four modalities of STDP (with permission from Feldman, 2012)**

STDP is prevalent across sensory systems, including the visual, entorhinal (Haas, Nowotny, & Abarbanel, 2006), auditory (Tzounopoulos, Kim, Oertel, & Trussell, 2004; Tzounopoulos, Rubio, Keen, & Trussell, 2007); and somatosensory system (Chou, Bucci, & Krichmar, 2015) (Figure 8). In the auditory system, a variety of learning rules reflecting STDP have been observed in the DCN, IC, and cortex (Figure 8). Specifically, Hebbian STDP is found

to be existent in excitatory neurons (i.e., fusiform cell), while anti-Hebbian STDP is in inhibitory interneurons (i.e., cartwheel cell) in the dorsal cochlear nuclei (Figure 8) (Tzounopoulos et al., 2004). Therefore, STDP rules vary with the types of post-synaptic synapses.

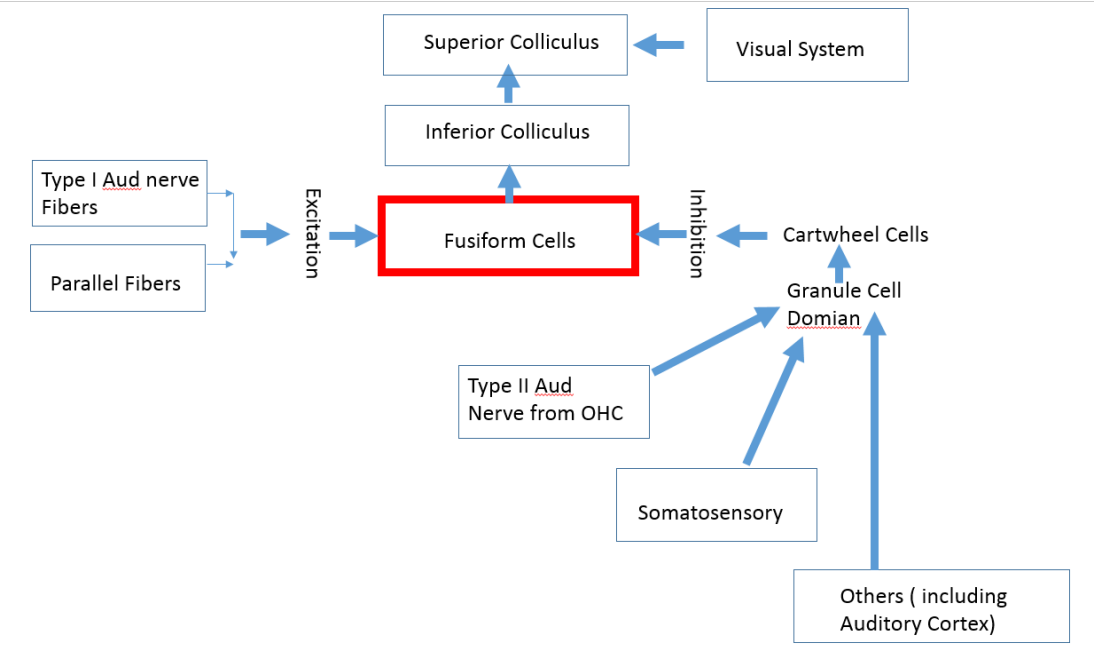



**Figure 8.The synaptic inhibition pathway from DCN to Superior Colliculi with the involvement of auditory, somatosensory and visual systems (derived from Textbook of tinnitus, 2011)**

Additionally, STDP can be manipulated by dendritic depolarization and neuromodulator induced inhibition, both of which can convert Hebbian STDP to anti-Hebbian STDP depolarization (Fino, 2005; Letzkus et al., 2006; Zhang, Lau, & Bi, 2009; Zhao & Tzounopoulos, 2011; Zilberter et al., 2009).

The effect of Hebbian and anti-Hebbian can be summed up and produces a net outcome of either LTP or LTD (Figure 7), depending on the order of EPSP spikes and post-synaptic spikes,

the timing, and weight of EPSP and post-synaptic spikes, as well as the cell membrane depolarization level (Tzounopoulos et al., 2004).

Synaptic cooperativity, multi-spike paring, and nonlinear summation of plasticity in natural spike trains are also factors that manipulate STDP (Clopath & Gerstner, 2010; Elstrott & Feller, 2009; Sjöström et al., 2001; Stuart & Häusser, 2001). Some of these factors can be the dominant ones for STDP formation in some circumstances but might only play minor roles in other conditions.

### 4.3 Summary of Tinnitus Generation/Persistence Loci and Triggers of Tinnitus

Here we briefly summarize the theories on the location of tinnitus generation and persistence, as well as the triggers of tinnitus inside of the auditory system. Concerning the location, the DCN has been identified as one site responsible for tinnitus generation but not tinnitus persistence. There is neither broad consensus as to the other loci responsible for tinnitus generation, nor the loci for tinnitus persistence. In general, tinnitus seems able to originate peripherally, centrally, or both, which likely varies depending on the etiology. In terms of the triggers of tinnitus, they could be classified into the category of deafferentation triggers and the category of non-deafferentation triggers. SFR and neural synchrony change would both lead to central gain enhancement, which is the most commonly accepted theory behind the sensory deafferentation triggered tinnitus. On the other end, excitotoxicity and activity-dependent plasticity are the hypothesized reasons behind the non-deafferentation based tinnitus.

These findings were established based on a combination of behavioral observation (i.e., lever press for food upon certain auditory stimuli) and electrophysiological activity (Brozoski & Bauer, 2005). Since this behavioral observation depends on several 10~15 weeks of training with animals, some researchers abandoned the behavioral observation, but depend entirely on the electrophysiological activity change after drug injection (Cazals et al., 1998; Guitton et al., 2003), anatomic ablation (Bledsoe et al., 2009) or noise trauma (Noreña & Eggermont, 2003). For example, Cazals et al. (1998) used salicylate and monitor the acute effects of salicylate injection on the auditory electrophysiological activity changes. While at the same time, they also might use lidocaine to those salicylates-injected guinea pigs, to see the difference of the effects on compound action potentials and electrophysiological cochleoneural activity between the salicylate + lidocaine vs. salicylate only situations. Salicylate is used because it can induce tinnitus in humans. At the same time, lidocaine can temporarily alleviate tinnitus in humans. They designed their animal model in this way, hypothesizing that the salicylate only animals would be those having tinnitus.

In contrast, salicylate + lidocaine animals would be the no-tinnitus group. However, there was neither a way to make sure how many animals started to have tinnitus because of the injection of salicylate nor a way to ensure how much the animals' tinnitus was alleviated because of the use of lidocaine. Therefore, even if they observed electrophysiological activity differences between the two conditions, no one could guarantee that those changes were due to tinnitus and not other auditory phenomena such as hyperacusis, misophonia, or auditory physiological change such as a slight synaptic transmission disturbance which might not be severe enough to induce tinnitus.

To summarize, there are well-constructed hypotheses and theories to test related to the loci of tinnitus generation and the neurophysiological mechanisms for tinnitus generation, and these remarkable findings are primarily rooted in animal studies. Nevertheless, based on the above

arguments, we can tell that the neuroscientific research of tinnitus cannot advance confidently without a method of verifying that tinnitus perception can accurately be identified in an animal.

## 5.0 Etiology of Tinnitus

While little consensus has been reached regarding tinnitus generation loci and tinnitus generation mechanisms, the etiology of tinnitus further complicates the question (Table 2) (Noreña & Eggermont, 2003).

Noise and multiple drugs are both associated with tinnitus. Salicylate, quinine, along with platinum-based anticancer drugs, are the well-studied drugs that can trigger tinnitus. Salicylate administration induces SFR increase at the IC and auditory cortex, but no change in the cochlea (Ma et al., 2006; Müller, Klinke, Arnold, & Oestreicher, 2003; Stypulkowski, 1990; Su, Luo, Wang, & Chen, 2009; Sun et al., 2009). Salicylate and noise may induce gain enhancement in the auditory cortex similarly (Noreña, Moffat, Blanc, Pezard, & Cazals, 2010). Still, salicylate caused tinnitus is reversible (Auerbach, Rodrigues, & Salvi, 2014). Mild to moderate doses of platinum-based drugs induced tinnitus by killing a certain amount of IHC while sparing OHC (Salvi et al., 2017). Interestingly, a compound action potential (CAP) amplitude reduction due to IHC loss is observed. Still, only a small reduction in IC and a robust neural enhancement in AC are reported (Wang et al., 1997). These effects suggest an increase of central gain, which is related to the lack of sensory input provided by selective damage to IHCs. Although noise-exposure often causes trauma to both IHC and OHC, the noise damage can be limited to OHC while sparing IHC as well, which is similar to some of the platinum-based animal studies. It shows that noise-induced cochlear damage that is limited to OHC does not change the cochlear nerve SFR (Dallos & Harris, 1978). Unlike OHC damage, IHC damage co-occurs with a drastic reduction of SFR in cochlea but increased at VCN, DCN, IC and auditory cortex (Brozoski & Bauer, 2005; Cai, Ma, & Young,

2009; Heinz, 2003; Kujawa et al., 2009; Liberman & Dodds, 1984; Noreña et al., 2010; Qiu, Salvi, Ding, & Burkard, 2000). The above findings confirm that a central gain can be induced by the lack of sensory input due to selective damage to IHCs, regardless of whether the damage was caused by drug or noise.

Although quite a bit about tinnitus etiology has been uncovered, much about tinnitus etiology is still not known. For instance, salicylate tends to reliably induce tinnitus, while limited studies showed that quinine's tinnitus effect depends on the route of quinine administration and the dosage of quinine (Jastreboff et al., 1991; Ralli et al., 2010). It remains unclear, which is the best administration and dosage for quinine to induce tinnitus in different animals. We may sometimes face a situation where testing parameters are almost identical between the two studies. Still, the study results are very different, which might potentially be due to different effects from various administrative paths and dosages. Moreover, although the IHC loss caused by platinum-based drugs is stable staying at 30~40% across animals, the gain enhancement varied substantially, which suggests the variability in central gain changes across animals is not only because of peripheral impairment differences but also seems to depend on several unclear factors in the central auditory system (Salvi et al., 2017).

For clinicians, an appropriate diagnostic and treatment strategy for tinnitus always begins with identifying the etiology of this auditory phenomenon. For animal researchers, the etiology of tinnitus is of interest not only because the different factors may explain some of the heterogeneity but because in the animal model, it is necessary to cause tinnitus using an outside agent. No outside agent produces tinnitus 100% of the time, which underscores the dilemma of being able to verify that the animal perceives tinnitus. However, since plenty about tinnitus etiology remains unknown, there needs to be a way to know when an animal has tinnitus.

**Table 2. Some common tinnitus etiology, related cochlear impairment, and relation to SFR change**

| *Common Tinnitus Etiology* | *Cochlear Impairment* | *Which impairment leads to SFR change* |
|---|---|---|
| Platinum-based anticancer drugs | (mild to moderate dosages) IHC only; (large dosage) OHC and IHC | IHC only; IHC + OHC |
| Salicylate and quinine | Both OHC and IHC | IHC + OHC |
| Noise trauma | Both OHC and IHC | IHC + OHC |

## 5.1 Summary of Etiology of Tinnitus

Researchers are equipped with rational hypotheses and theories of the loci of tinnitus generation and have several agents that can be administered in the animal model to induce tinnitus. The challenge remains as to how one verifies that noise or drug administration has resulted in tinnitus in an animal. The next section reviews a specific paradigm that has been proposed to test tinnitus in the animal model, and that might be conducive to validating tinnitus in human subjects.

## 6.0 Is Tinnitus Filling in the Gap?

Animal studies using two test paradigms that have been developed to identify if an animal is experiencing tinnitus will be reviewed. Human tinnitus researchers are interested in these objective measuring paradigms developed for evaluations of animals. There is disagreement surrounding whether these paradigms are identifying tinnitus and, if they do, what underlying mechanisms are supporting these evaluations. Human studies also are reviewed with attention to methodological issues.

## 6.1 SPIAS and GPIAS

### 6.1.1 A Brief History of GPIAS and Its Related Tinnitus Measurement Methods in Animals

Gap-Prepulse Inhibition of Acoustic Startle (GPIAS) was initially proposed by Turner et al. (2006) as a novel model for tinnitus assessment in animals. Before the GPIAS method, Bauer and Brozoski's (2001) method had been most commonly used (Bauer & Brozoski, 2001).

*Bauer and Brozoski's method*

In Bauer and Brozoski's method, rats are initially trained continuously for several days/weeks to lever-press for food pellets upon hearing sound signals. The rats are trained for 60 minutes per day. Once these rats are pressing reliably, they are placed on a variable-interval schedule, and the interval is gradually increased. Variable interval schedules are reinforced to reach a relatively stable response rate with very low within-subject variability. In order to explore

the effect of unilateral noise trauma level on tone perception, animals are exposed to 0, 1, or 2 hours of unilateral noise trauma. It is believed by Bauer and Brozoski that the shift in the discrimination functions produced by unilateral trauma was frequency-specific, as would be predicted by tonal tinnitus but not by simple hearing loss. That being said, animals without tinnitus but with only a unilateral hearing loss should display a very different shift in their discrimination functions than that exhibited by animals with tonal tinnitus. This assumption is essential because a tinnitus explanation becomes unnecessary if unilateral hearing loss produced the same shift in psychophysical functions as that obtained following unilateral trauma.

The establishment of GPIAS as a tinnitus testing model is based on the validity of Bauer and Brozoski's method; for that reason, it is critical to understand whether Bauer and Brozoski's method truly measures tinnitus. This method is looking at the lever-pressing rates of tested animals upon hearing signals (i.e., pure tones, noise, and no sound). Animals were supposed to press a lever for food when hearing the correct stimuli, and incorrectly pressing the lever would lead to a shock as punishment. For example, animals with 20 kHz tonal tinnitus would be assumed to press the lever incorrectly more often than their peers without 20 kHz tonal tinnitus if the correct stimuli were set as the 20 kHz pure tones. The correct lever-pressing rate was considered as a reflection of auditory psychophysical function. It, therefore, was given a score for such function to show whether there was a statistical difference between a group with 2 hours of unilateral broadband noise (BBN) exposure, a group with 1 hour of unilateral BBN, and two control groups that are without any noise exposure. The two control groups were: the control with no earplug group and the control with the earplug group. To clarify, the intensity of BBN was 60 dB SPL in their study. All four groups were measured with click ABR, and tone burst ABR before and after noise exposure (Table 3).

**Table 3. Threshold elevation after noise exposure or using an earplug**

| ***Groups*** | 1 hour traumatized group | 2 hour traumatized group | Controls with no ear plug group | Controls with ear plug group |
|---|---|---|---|---|
| ***Threshold elevation after noise exposure or using ear plug*** | 60 dB SPL elevation across 4 KHz to 31.5 KHz due to noise exposure | 60 dB SPL elevation across 4 KHz to 31.5 KHz due to noise exposure | (no noise exposure) no elevation | (no noise exposure) 40 dB SPL elevation across 4 KHz to 31.5 KHz due to ear plug |

All four groups were tested with tones across frequencies. The 1-hour traumatized group showed significantly lower psychophysical function scores compared to the controls with and without earplug groups, with the most considerable difference at 20 kHz precisely. Controls with earplugs (i.e., the group with unilateral hearing loss only) and controls with no earplugs showed no significantly different function scores between each other when tested with tones at various frequencies. Therefore, the authors believed that the result from the 1-hour traumatized group could not be explained by hearing loss and provided strong evidence of the existence of 20 kHz tonal tinnitus in that group. In contrast, the 2-hour traumatized group showed significantly lower scores compared to both control groups as well, but the difference was across all tested frequencies. Thus, the authors concluded that the longer (i.e., 2 hours) noise exposure induced broadband tinnitus rather than tonal tinnitus.

However, because both 1-hour and 2-hour traumatized groups showed 60 dB SPL elevation of hearing threshold across frequencies in their noise-exposed ears, while the control with earplug group showed 40 dB SPL elevation of hearing threshold across frequencies, the results might not be exclusively explained by tinnitus. The results might also partially be explained by the extra 20 dB SPL of threshold elevation. Additionally, since both 1-hour and 2-hour traumatized groups showed significantly lower psychophysical function scores across frequencies compared to both

control groups, it might not be appropriate to conclude that 1-hour BBN noise exposure led to tonal tinnitus and 2-hour BBN noise exposure led to broadband tinnitus. Therefore, their conclusion of tonal and broadband tinnitus might be misleading. Because of this, the subsequent findings made by researchers who use the Baur and Brozoski method to test tinnitus, especially the tonal tinnitus, are called into question.

*GPIAS and its relationship with Baur and Brozoski's method*

Psychology studies reveal that a gap in an ongoing sound just before a loud startle stimulus is introduced decreases the amplitude of the startle reflex as measured by an electrophysiological response. Such gap induced startle response reduction is called a Gap-Prepulse Inhibited Acoustic Startle (GPIAS) reflex response. It is believed by animal researchers that the startle reflex response would remain the same between conditions with and without gap-prepulse in rats with tinnitus because tinnitus is filling in the gap that is introduced to the ongoing sound being presented to the listener. So, in the GPIAS method, the testing is set up in a way that can catch the instant startle response from an animal. Rats remained in a small testing chamber that has limited space so that the rats cannot move around easily and, therefore, maximally controls the moving-related artifacts. Startle stimuli are presented through a speaker located in the ceiling of the testing chamber, above the rat's head. The floor of the chamber is attached to a pressure transducer, which receives, and measures startle force applied to the floor. Background sound in the startle chamber is provided with gaps embedded. In GPIAS testing, the rats are not required to have any food or water deprivation, no training, learning, and motivational demand. They just stay inside of the testing chamber and are exposed to the gap embedded background sound along with a sudden startle-eliciting noise burst. The GPIAS testing can be completed in about 40 minutes, and the startle

response transduced from the floor of the chamber is automatically collected by commercialized animal startle reflex hardware.

Bauer and Brozoski's method has the advantage of relatively high test-retest reliability. Still, it has the disadvantage of the duration of animal training time and the control of natural aging effects in animals, especially young rodents. In contrast, the GPIAS model does not require any behavioral training or food/water deprivation for animals; therefore, it is easy to manage. Additionally, testing can be done quickly, allowing an instant indirect measurement of tinnitus resulting from acute noise or drug exposure. For these reasons, the GPIAS model has gradually become the most popular method of testing tinnitus in animals.

Can noise or ototoxic drug exposure lead to tonal or frequency-specific tinnitus in animals? Regarding this question, Turner (2006) used both the GPIAS paradigm and Bauer and Brozoski's method, setting the later one as a gold-standard method to confirm noise trauma-induced tinnitus perceptions like a frequency-specific tone at 10 kHz. What they found was that the psychophysical function deficit at 10 kHz based on Bauer and Brozoski 's method matched with the lack of startle inhibition at 10 kHz. Thus, Turner (2006) concluded that GPIAS is an easier and more efficient method of testing tinnitus in animals compared to Bauer and Brozoski's method. Grounded in this conclusion, Turner moved forward and found that animals with salicylate exposure also had frequency-specific lack of startle inhibition, which were attributed by the authors as a reflection of tonal tinnitus (Turner & Parrish, 2008). A lack of gap-repulse induced acoustic startle inhibition means that the startle reflex was not inhibited with the presence of a gap in the ongoing background sound prior to the startle stimulus being presented. The hypothesis is that the animal experiencing tinnitus did not experience the gap because they continued to hear their tinnitus during this time. Therefore, a reduction in startle reflex would not be expected since the experience of the gap in

the ongoing sound is what produces the reduction in a startle. However, as mentioned earlier, whether GPIAS exclusively measured tinnitus remains ambiguous because the GPIAS is compared to the method of Baur and Brozoski method, which might not be an exclusive reflection of tinnitus without excluding other factors, such as threshold shift. Moreover, the mechanism behind the lack of gap-prepulse induced acoustic startle inhibitions phenomenon in Turner's study remains ambiguous.

### 6.1.2 The Testing Procedure of SPIAS and GPIAS

Like SPIAS, the GPIAS is a reflection of an automatic process at the pre-attentive stage (Cromwell, Mears, Wan, & Boutros, 2008). The combined SPIAS/GPIAS has been proposed as a method to identify if an animal is experiencing tinnitus indirectly. The overarching concept is that if the animal perceives sound all the time (e.g., tinnitus), then that will alter their startle reflex in a known way. The testing procedures and parameters of GPIAS are similar to those of SPIAS. For example, SPIAS testing is done in a quiet background, and a 50 milliseconds duration prepulse stimuli at 55 dB SPL is given several milliseconds before the startle stimulus (Figure 9). In GPIAS testing, instead of providing a 50-milliseconds duration sound prepulse stimuli in a quiet background, a 50 milliseconds duration silent gap is presented in continuous background sound (Figure 9). If the animal is experiencing tinnitus, the theory is that there will not be a silent gap because the tinnitus fills in the gap. As a result, the startle will be less reduced compared to either the reduction level before noise/drug exposure or the reduction level of animals in the non-exposure control group (Figure 10). Some researchers use SPIAS and GPIAS together in animals, so that a change in GPIAS is not believed to be related to a temporal processing or sensorimotor

deficit, but rather related to other reasons such as tinnitus if SPIAS remains unchanged. At the same time, GPIAS shows a negative change after noise or drug exposure.

In animal studies, these SPIAS and GPIAS measurements include placing the animal inside an acoustic chamber (Figure 11). Within the chamber, a transparent tube is positioned at a specific distance from a loudspeaker that is in front of the animal (Ahlf, Tziridis, Korn, Strohmeyer, & Schulze, 2012; Tziridis et al., 2015). The animals are put inside the tube. The startle response is measured by a force sensor attached underneath the tube, as mentioned in an earlier section of this manuscript.

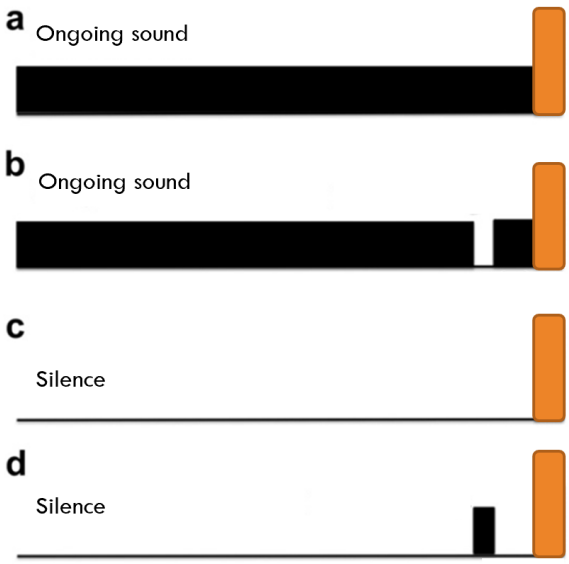



**Figure 9.SPIAS and GPIAS. The orange bar indicates the startle stimulus. Panel a: the startle only condition for GPIAS paradigm is essentially a no-gap condition. The startle only condition for GPIAS involves an ongoing sound, followed with a startle stimulus at the end. Panel b: the prepulse condition for GPIAS involves a gap embedded ongoing sound, followed with a startle stimulus at the end. Panel c: the startle only condition for SPIAS paradigm involves a period of silence, followed with a startle stimulus at the end of the**

**silence. Panel d: the prepulse condition for the SPIAS paradigm involves a period of silence, followed with a startle stimulus at the end, and a sound prepulse placed before the startle stimulus.**

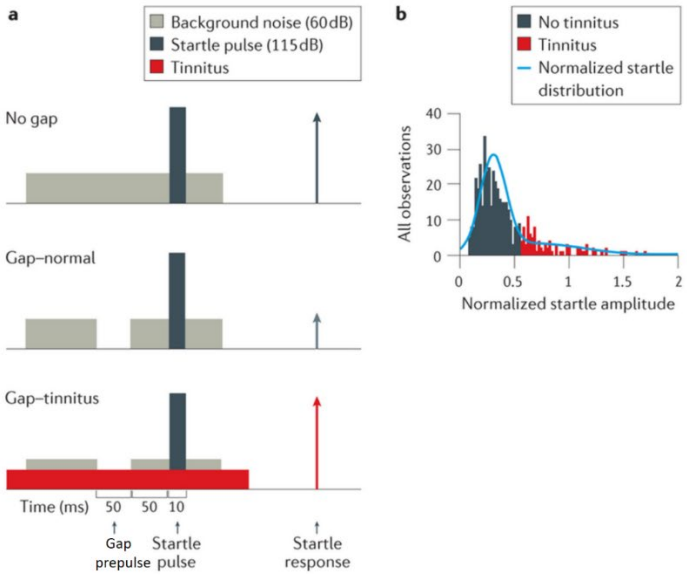



**Figure 10.A. Schematic describing the GPIAS paradigm for tinnitus. B. Histogram of the normalized startle distribution (white line) partitioned into two distributions: no evidence for tinnitus (black bars) and evidence for tinnitus (red bars) (with permission from Shore, 2016)**

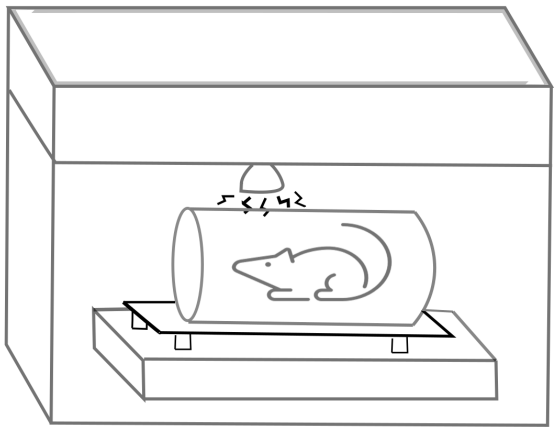


**Figure 11.An illustration of an animal startle response system**

### 6.1.3 The Relationship between SPIAS and GPIAS

SPIAS is a measure of temporal processing and sensorimotor gating ability (Braff & Geyer, 1990; Geyer & Braff, 1987; Moran, Booze, & Mactutus, 2013). The combination of SPIAS and GPIAS in tinnitus study seems sensible since animals that pass SPIAS testing are thought to be able to hear the sound-prepulse/background sound. Those animals that have a lack of inhibition in GPIAS testing but not in SPIAS testing are deemed to have tinnitus ( Dehmel, Eisinger, & Shore, 2012; Koehler, Pradhan, Manis, & Shore, 2011; Llano, Turner, & Caspary, 2012; Turner et al., 2006). Of note, studies have only used gap-prepulse or sound-prepulse with at least 20 ms durations; thus, these findings can only support the conclusion that an individual with normal SPIAS has intact temporal processing and sensorimotor gating ability when the duration of prepulse is at least 20 ms.

Some researchers believe that SPIAS is simply the inverse of GPIAS (Dehmel, Eisinger, & Shore, 2012; Longenecker, Alghamdi, Rosen, & Galazyuk, 2016; Longenecker, Chonko, Maricich, & Galazyuk, 2014). However, this assumption is not accurate. First, if SPIAS could just be deemed as the inverse of GPIAS, the startle reflex suppression level would be similar. However, in reality, SPI is much more robust than GPI in both exposed and non-exposed groups, which indicates a different amount of suppression between SPIAS and GPIAS (Fournier & Hébert, 2013). Second, although the neural circuit of SPIAS is better studied and understood than that of GPIAS, the limited evidence shows different neural circuits of these two testing conditions (Leggett, Mendis, & Wham, 2018). The neural circuitry mediating startle is short, as indicated in Figure 12. The startle reflex can be elicited from the trigeminal nuclei, cochlear nuclei, or vestibular nuclei. The stimulation is further projected to the caudal pontine reticular nucleus (PnC), which also funnels neural activity to motor areas of cranial nerve nuclei and the spinal cord (Koch & Schnitzler, 1997; Yeomans, Li, Scott, & Frankland, 2002).

The primary circuitry mediating SPIAS is located in the brainstem including the inferior colliculi (IC), superior colliculi (SC) and pedunculopontine tegmental nucleus (PPIg) (Kodsi & Swerdlow, 1997; Leitner & Cohen, 1985; Yeomans, Lee, Yeomans, Steidl, & Li, 2006). Newer studies are challenging the traditional view by showing SPIAS without SC; thus, Li et al. (2009) suggest that there might be more than one pathway mediating SPIAS. This pre-attentive sensory gating can be driven by top-down activity, proved by both human and animal studies (Li et al., 2009; Norris & Blumenthal, 1996)(Figure 13-14). Besides, the anticipation of affective stimuli draws more attentional resources than neutral stimuli and results in an augmented SPIAS effect (Li et al., 2009). Human subjects with tinnitus with high anticipation of upcoming stimuli,

therefore, will quite possibly have more significant prepulse inhibition when hearing tinnitus like an auditory stimulus in the experiment.

SPIAS is believed to be an operational measure for sensorimotor gating and sub-cortical phenomenon, but this is not to say that SPIAS cannot be modulated through top-down projections from the auditory cortex and non-auditory cortex (Figure 13-14). Deficient SPIAS responses have been observed in patients who failed to filter cognitive, emotional, and primary insomnia (Braff et al., 1978; Grillon, Morgan, Southwick, Davis, & Charney, 1996; Petrovsky et al., 2014). SPIAS can be enhanced with attention in both animal and human subjects, possibly via the corticofugal pathway (Filion & Poje, 2003; Heekeren, Meincke, Geyer, & Gouzoulis-Mayfrank, 2004; Röskam & Koch, 2006; Srinivasan, Keil, Stratsis, Woodruff Carr, & Smith, 2012) (Figure 13). Although SPIAS effects can be enhanced with cortical involvement, the cortex is not a necessity (Bowen, Lin, Taylor, & Ison, 2003; Du, Wu, & Li, 2011; Eggermont, 2013; Hunter & Willott, 1993; Threlkeld, Penley, Rosen, & Fitch, 2008).

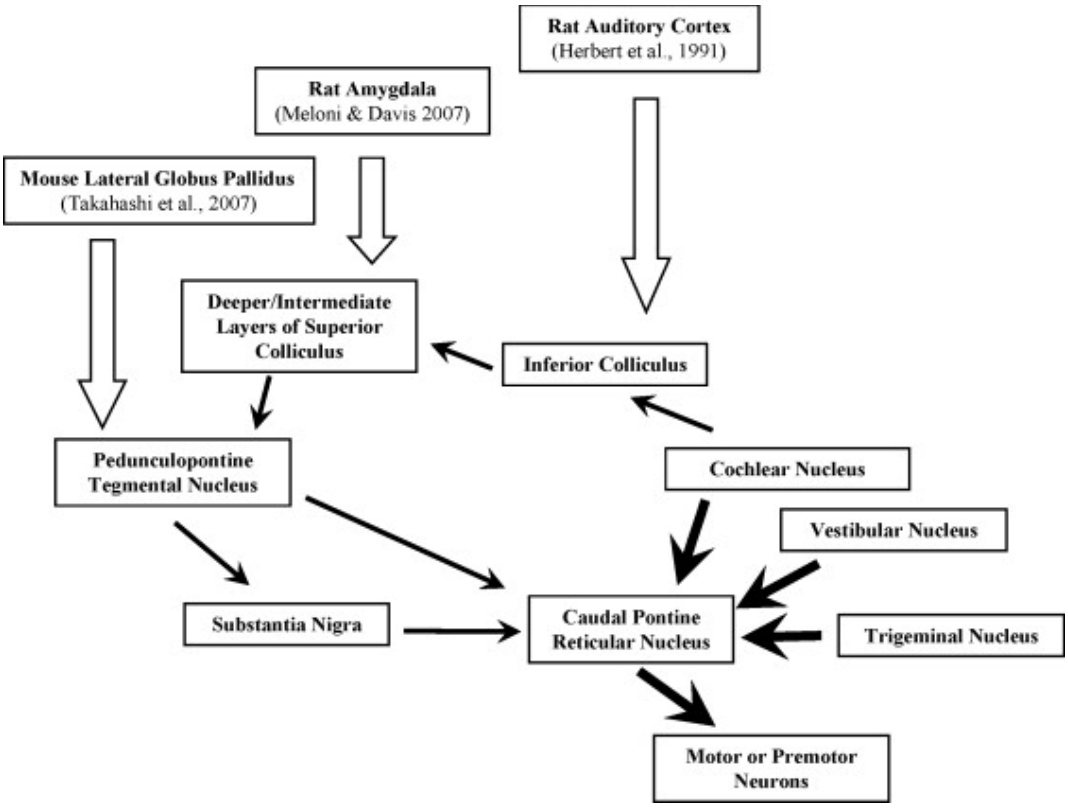



**Figure 12.Neural pathways mediating (1) startle responses (thick black arrows), (2) auditory prepulse inhibition (thin black arrows), and (3) modulation of prepulse inhibition (thick unfilled arrows). (with permission from Li, 2009). Of note, the citation of "Meloni & Davis 2007" in this original graph is incorrect. It should be "Meloni & Davis 2000".**

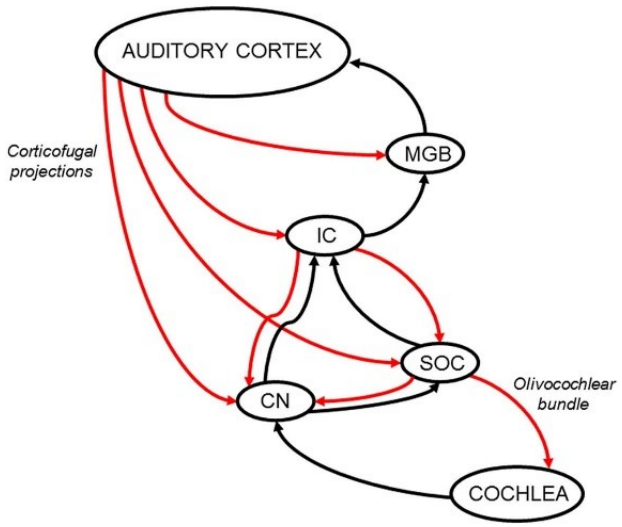



**Figure 13.Schematic diagram of the auditory efferent network (with permission from Terreros, 2015). Corticofugal projections from the auditory cortex to the inferior colliculus (IC) and medial geniculate body (MGB) and afferent connections from the IC and MGB to the auditory cortex form a colliculi-thalamic-cortical-colliculi loop within this network.**

Unlike SPIAS, the neural circuitry controlling GPIAS is poorly understood. A recent finding indicates that although sound prepulse and gap prepulse can both efficiently suppress the acoustic startle reflex, the inhibition of the acoustic startle response by prepulse or gap is orchestrated by different neural pathways since sound prepulse is activating the lateral globus pallidus (LGP) to inhibit the startle response. In contrast, gaps bypass the LGP to activate the auditory cortex (Moreno-Paublete, Canlon, & Cederroth, 2017).

A lack of gap-prepulse inhibited acoustic startle inhibition is associated with changes in neural synchrony and SFR (Dehmel, Pradhan, Koehler, Bledsoe, & Shore, 2012). However, even with a well-controlled hearing threshold and evidence of increased neural synchrony and SFR, we still cannot guarantee that such frequency-specific lack of gap induced startle inhibition is a reflection of the tinnitus frequency. In order to fill the silent gap in the GPIAS paradigm, the

tinnitus frequency should be flawlessly matched to the background sound during the test, since unmatched frequency can be an even more salient hint than intensity in gap detection. However, it is currently impossible to match tinnitus pitch and loudness in animals because animals cannot directly communicate subjective auditory experiences with researchers.

### 6.1.4 A Summary of SPIAS and GPIAS

Although acoustic startle is a sub-cortical response, GPIAS is more affected by cortical activity than SPIAS. Researchers have been combining both SPIAS and GPIAS to study tinnitus in animals. The assumption behind the combination is that if animals have no SPIAS change but a GPIAS reduction after noise trauma, then the GPIAS weakness is due to other acoustic issues rather than hearing loss since there is no sensory and motor gating defect. They assume the inhibition deficit is a result of tinnitus because tinnitus fills in the gap.

Although it is reasonable to hypothesize that such a frequency-specific lack of gap induced startle inhibition might be reflecting a tinnitus frequency or tinnitus filling in the gap, this hypothesis has not been proved with strong evidence in animals due to the natural limitation with animals in that they cannot communicate their subjective perception of tinnitus with researchers. It is plausible that lack of gap-prepulse inhibited acoustic startle inhibition is a reflection of hidden hearing loss, hyperacusis, or both, rather than tinnitus per se. Because hearing loss can affect the audibility in GPIAS tests and thus alter the results, for instance, animals with hearing loss cannot hear the ongoing background sound. Therefore the silent gaps might be easily missed in the gap condition. While in no-gap condition, the poor audibility of the ongoing background sound may make the animals feel there is only a startle stimulus alone. However, there is a continuous

background sound existent before the startle stimulus. The under-awareness of the ongoing background sound may potentially alter the amplitude of the startle reflex.

Additionally, hyperacusis has a high co-morbidity with tinnitus in humans. Hyperacusis is believed to be closely linked to fear and other negative emotional feelings. Aversive emotion magnifies the startle reflex, and therefore hyperacusis is another confounder that needs to be controlled in the GPIAS test (Essex et al., 2003; Waters, Lipp, & Spence, 2004). Along with the issue of hearing loss and hyperacusis, the theory of tinnitus filling in the gap in the GPIAS paradigm has been most criticized. Human research is needed to validate the speculative theory of tinnitus filling in the gap since participants can report that they have tinnitus and characterize the percept.

### 6.2 Relationship between GIN and GPIAS

The gaps in noise (GIN) test procedure does not contain a startle response, which is quite different from the procedures of SPIAS and GPIAS. The GIN test is composed of a series of 6 seconds broadband noise segments. Each segment of noise contains 0 to 3 silent gaps. Each gap lasts in duration from 2 to 20 milliseconds. The GIN is presented monaurally, and the subjects are instructed to push a response button as soon as they hear a gap. For example, if a subject hears two gaps in one noise segment, then he is supposed to push the button twice during that segment of the test.

In addition to the different testing procedures, there are other differences between GIN and GPIAS. The most significant difference between the two tests is that conscious attention/active

listening is required in GIN since it is a perceptual task (Table 4), while GPIAS is a sensorimotor gating process that presumably involves pre-attentive filtering of sensory stimuli (Cromwell et al., 2008; Geyer, 2006). Although GPIAS can be observed in animals with surgically or chemically suppressed cortical function, several studies have shown that the auditory cortex may be involved in regulating GPIAS when the gap is short enough, such as 2~20ms (Silverstein, Graham, & Calloway, 1980; Threlkeld et al., 2008; Weible et al., 2014). Additionally, although attention can enhance the acoustic startle inhibition at the sub-cortical level, attention likely compensates the sub-cortical inhibition deficit at the level of the cortex (Filion, Dawson, & Schell, 1993) (Figure 12-13). Although both can be associated with cortex function to some extent, GIN performance appears to be non-equivalent to GPIAS performance. GPIAS requires an automatic, involuntary reflex, which involves a shorter neural circuit, while GIN requires subjects to give answers for what they hear, which is a non-automatic voluntary activity.

It is now clear that GIN and GPIAS are two different physical phenomena; the following section will then focus on explaining whether the lack of startle inhibition from the GPIAS paradigm is the same as a gap detection deficit from the GIN test.

### 6.2.1 Is lack of Gap Induced Acoustic Inhibition Due to GIN Deficit?

The answer is not entirely definite, but some evidence shows that the lack of gap induced acoustic startle inhibition might not be due to the GIN deficit in subjects with tinnitus. Three studies of active listening GIN in tinnitus and one study of passive listening GIN in tinnitus measured by EEG (Table 4) were conducted. Among the three GIN studies involving active listening, Gilani et al. found a GIN deficit in subjects with tinnitus, the other two studies from

Campolo et al. and Boyen et al. showed no GIN deficits in subjects with tinnitus (Boyen, Başkent, & Van Dijk, 2015; Campolo, Lobarinas, & Salvi, 2013; Gilani, Ruzbahani, Mahdi, & Amali, 2013). Although both Boyen and Campolo drew the same conclusion that there is no GIN deficit in subjects with tinnitus, several differences between the two studies should be noted. Firstly, Boyen did a sex/gender match, but Campolo did not. Secondly, Campolo did a thorough hearing threshold measure, including extra high frequencies, and also matched the subject's tinnitus pitch with background sound in the GIN test, but Boyen did not. Campolo's study is the only study that matched the subjects' tinnitus pitch with the background sound pitch among all human GPIAS/SPIAS/GIN studies. Thirdly, Boyen tested a shorter gap duration (<30 ms) while Campolo tested only with a 50 ms gap. Thus, Campolo et al. possibly failed to see any difference because of the floor effect, with GIN being too easy for their study design. While Campolo provided proper matching, and Boyen did some matching before 8 kHz, Gilani only used the original GIN test developed by Musiek without making any modifications, such as background sound with tinnitus pitch matched (Musiek et al., 2005) (Table 4). Therefore, although Gilani found significant GIN deficits in the tinnitus group, the results and conclusions remain unclear. Both studies from Boyen and Campolo did not see GIN differences between the tinnitus group and the control group. Boyen suggested that their GIN test may still be too easy to show any gap detection deficit in people with tinnitus. Gilani had gaps with 2~20 ms durations, which is much shorter than the gap durations in studies from Boyan and Campolo. Another possibility for differences in results could be hearing loss since both Boyen and Campolo did not exclude subjects with hearing loss. At the same time, Gilani and Campolo only included people with normal hearing.

To summarize, there is no supporting evidence to show GIN deficits in individuals with tinnitus when gap durations are over 20 ms based on available evidence. The gap detection

threshold of GIN in naive adult listeners was about 4.9 ms, with a standard deviation of 1ms (Musiek et al., 2005). Thus, if there is any startle inhibition deficit based on the GPIAS test, it cannot be explained by the gap detection deficit based on the GIN test since all the GPIAS studies adopted >20ms gap durations.

### 6.3 GPIAS/SPIAS/GIN Studies in Humans

Three studies applied GPIAS to test tinnitus in human subjects. Although all are based on the same GPIAS paradigm, testing parameters, and conclusions varied (Table 4 & Table 5). In this section, attempts are made to quantify the results related to testing parameters and potential confounds across human GPIAS studies.

**Table 4. Pros and cons of human tinnitus studies using GPIAS/SPIAS/GIN**

| | GPIAS /SPIAS /GIN | well-controlled tinnitus pitch match with background sound in the test | well-controlled tinnitus type | Gap duration is short <20 ms/ task is difficult enough | sex and age-matched | the measured extra-high frequency range of hearing threshold | no hearing threshold difference between tinnitus group and control group | the gap-startle stimuli interval is ≈120ms | SPIAS/GPIAS/GIN | Passive listening /attention not required |
|---|---|---|---|---|---|---|---|---|---|---|
| Fournier 2013 | SPIAS & GPIAS | NO | YES | NO | NO | YES | YES | YES | SPIAS &GPIAS | NO |
| Ku 2017 | GPIAS | YES* | NO | NO | YES | NO | NO | YES | GPIAS | YES |
| Shadwick 2014 | GPIAS | NO | NO | NO | NO | NO | NO | NO | GPIAS | YES |
| Mahmoudian 2013 | GIN | NO | NO | YES | YES | NO | YES | NO | GIN | YES |
| Campolo 2013 | GIN | YES | NO | NO | NO | YES | NO | NO | GIN | NO |
| Gilani 2013 | GIN | NO | NO | YES | YES | NO | YES | NO | GIN+DPT | NO |
| Boyen 2015 | GIN | NO | NO | NO | YES | NO | NO | NO | GIN | NO |

- Note: Ku et al (2017) only included 8KHz tinnitus in their study, but lack of details on how they picked the 8kHz subjects with tinnitus

- DPT: duration pattern test

### 6.3.1 Gap Duration and Gap-Startle Stimuli Interval in GPIAS

Gap duration matters in GIN and should play a role in GPIAS as well. In Ku's (2017) study, only test sessions with 20 ms gap duration showed significant differences between subjects with tinnitus and controls when they tested with tinnitus frequency-matched background sound, while no group differences are found when they tested with 20 ms gap but non-matched frequency background sound, and also no group differences are found when they tested with 50 ms and 100 ms gap durations with both matched and non-matched frequency (Ku et al., 2017). Thus, Ku et al. suggested that the effect of tinnitus emerged depending on gap durations rather than tinnitus filling in the gap. Whether the tinnitus pitch and intensity are matched with the background sound also played a role in their results. Ku did not match the background sound intensity with the tinnitus

loudness intensity, which means when the noise stopped, the tinnitus may not have fully filled in the gap due to the intensity contrast. Will such contrast unconsciously bring any hint to the cortex and further affect the sub-cortical activity? The answer remains unclear so far. The role of the sound intensity match will be further discussed in the following section.

Additionally, we are not entirely clear of the role of the gap-startle stimuli interval in GPIAS. The lack of inhibition on startle response only occurred when the gap is placed within a specific range before the startle stimulus (Hickox & Liberman, 2014). Therefore Galyzyuk and Hebert believed that this gap-dependent phenomenon contradicts the tinnitus filling-in hypothesis since if this hypothesis is correct, then the occurrence of lack of gap induced startle inhibition should be independent of gap location (Galazyuk & Hébert, 2015). Nevertheless, rather than concluding that tinnitus is not filling in the gap, it would be safer to suggest that there might be a range for gap locations that are most sensitive to show the lack of gap-prepulse induced acoustic startle inhibition. In contrast, no lack of startle inhibition could be detected if the gap locations are out of that range. 120 ms has already been shown to maximize magnitude inhibition (Braff et al., 1978). In human GPIAS studies, Shadwick did not report their gap-startle stimuli intervals.

In contrast, Ku and Fournier reported gap-startle stimuli interval, which is close to 120 ms in their studies (i.e., 100ms in Ku's study and 120ms in Fournier's study). However, Shadwick did not find a lack of gap-prepulse induced startle inhibition in tinnitus groups, while Ku and Fournier did. These conflicts in study findings further suggest that the gap-startle stimuli interval might be a critical parameter for consistent GPIAS performance.

Some researchers employed GIN as a substitute for GPIAS in testing tinnitus in humans and tried to conclude whether tinnitus was filling in the gap based on their GIN results.

Among these GIN studies, only Mahmoudian and Gilani found a GIN deficit in the tinnitus group. None of them had an excellent tinnitus pitch and loudness match to the ongoing background sound that was used in the study, so none of their studies are suitable or qualified to support or negate the tinnitus filling in the gap theory. However, the continuity illusion (Figure 14) may explain the results in the Mahmoudian GIN study. The continuity illusion describes a phenomenon where a tone that is interrupted by a silent gap is perceived as discontinuous (Panel A in Figure 14). However, the tone may be perceived as continuing through a white noise when the gap is filled with this white noise (Panel B in Figure 14). This continuity illusion depends more or less on the masking of the absent target sound (Riecke, Van Opstal, & Formisano, 2008).

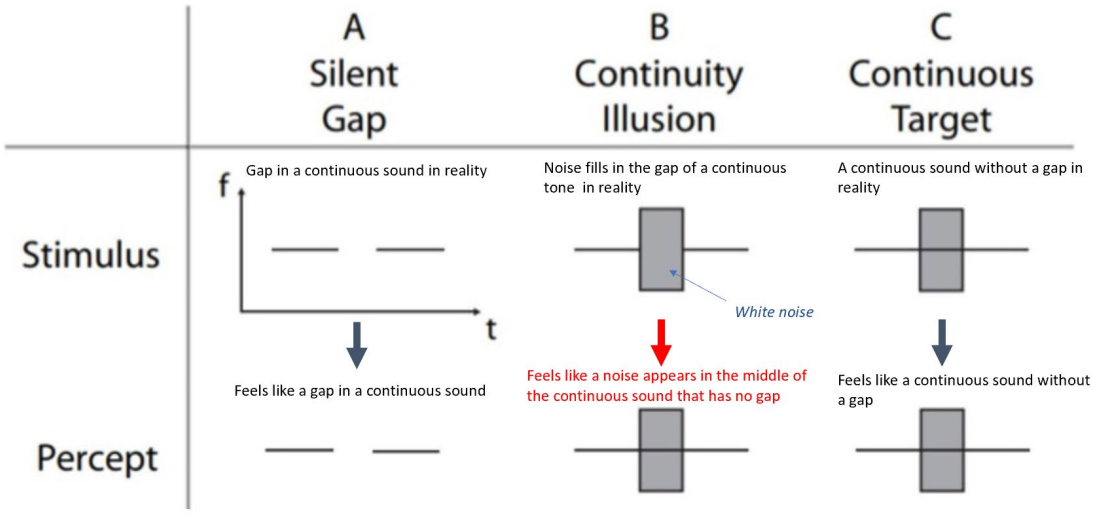



**Figure 14. The auditory continuity illusion exemplified for steady-state tones.**

(A) Two tones interrupted by a silent gap are perceived as two independent entities. (B) The illusion of a single entity when a broadband noise master is added to the gap, a percept that is similar to physically continuous tones, shown in Panel C. (with permission from Riecke et al., 2008)

The continuity illusion can explain Mahmoudian's GIN finding, and also explain Fourniers's GPIAS result since Fournier found a lack of startle inhibition with a 50 ms gap in the tinnitus group with both 500 Hz and 4 kHz background sound settings. At the same time, almost none of his subjects had 500 Hz tinnitus. Continuity illusion theory can explain the lack of startle inhibition in the tinnitus group of Fournier's study when subjects had tinnitus with drastically different central frequencies from that of the background sound being used in this study. However, continuity illusion cannot explain Ku's study, which found a lack of startle inhibition with a 20 ms gap but not the 50 or 100ms gaps. This theory cannot explain Ku's results, because as long as the gap duration is shorter than 900 ms, they should all be unable to perceive the gap across frequencies from 500 Hz to 6000 kHz based on continuity illusion (Figure 15). Continuity illusion does not have such sensitivity in small gap duration differences.

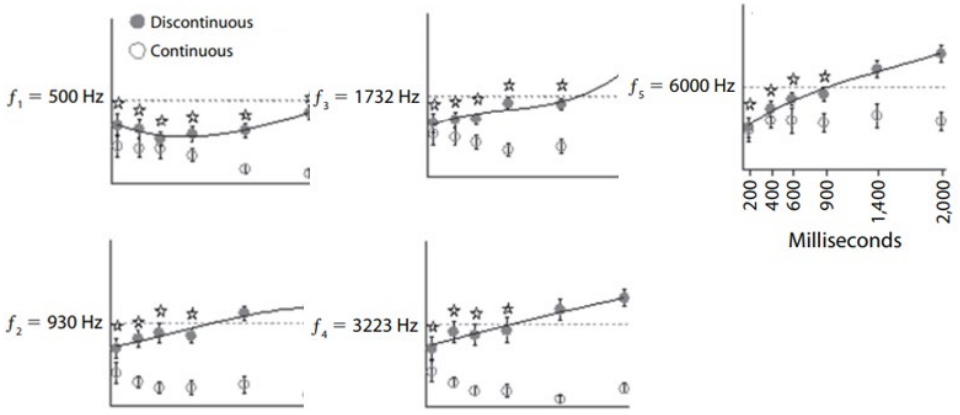



**Figure 15. Noise duration and Mean percept (+/- SEM) of experimental stimuli, representing discontinuous conditions (solid circles), and control stimuli, representing continuous conditions (open circles), per frequency across listeners (with permission from Riecke et al., 2008)**

### 6.3.2 Tinnitus to Background Sound Intensity and Frequency Match in GPIAS

In SPIAS, both pre-pulse sound and eliciting sound intensities together affect the startle response result, regardless of tinnitus (Blumenthal, 1996). Thus, it is reasonable to assume the gap intensity, which is an intensity contrast induced by the gap in the background sound, will affect the startle response (Blumenthal et al., 2005). In other words, the background sound intensity matters, even without considering the factor of tinnitus.

Regarding background sound intensity, all human studies met the criteria that the impulsive or impact noise should be at lower than the maximum limitation at 140 dB peak SPL to avoid potential harm according to the OSHA standard (Passchier-Vermeer & Passchier, 2000). However, no startle-noise intensity ratio has been set up as a relatively universal criterion. Berg (1973) reported a 50% probability threshold for a blink response is 85 dB(A) SPL. Hence, the intensity level of 100 dB(A) SPL or more is adopted by a high number of acoustic startle studies.

Pitch match between the ongoing background sound and tinnitus is another crucial factor that determines the validity of GPIAS studies. Among the three GPIAS human studies, only Ku's study matched the tinnitus pitch with ongoing background sound frequency. Ku only included subjects with 8 kHz tinnitus along with subjects with no tinnitus; however, they failed to explain how they selected the 8 kHz subjects with tinnitus and what kind of tinnitus measurement steps they completed. Fournier's and Shadwick's studies also had frequency matching issues. Fournier & Hébert (2013) used a startle acoustic (ocular EMG) embedded in their GPIAS paradigm in humans. This study showed a consistent startle inhibition deficit in participants with high-pitched tinnitus no matter the GPIAS test was conducted in ongoing background sound that had a relatively low or high central frequency. They believed that such results suggest that the tinnitus percept is

not filling in the gap and is unlikely to be responsible for lack of startle inhibition. However, this conclusion might be inappropriate because of poor pitch matching between tinnitus and background sound. In their study, most subjects reported tinnitus frequency ranging from 8kHz ~16kHz. Nevertheless, Fournier et al. only gave subjects two optional background sounds to be tested (i.e., 4kHz as a high-frequency sound group or 500 Hz as a low-frequency sound group). Interestingly, the authors found that the GPI is more significantly enhanced at 4kHz in subjects with tinnitus compared to the 500 Hz condition, which could be explained by the 4kHz being much closer to their tinnitus frequency than 500 Hz.

### 6.3.3 Cognition and Attention in GPIAS

Fournier's study is the only one among the three GPIAS human studies that required subjects' attention. Subjects were asked to listen actively to the background sound and startle stimuli. Subjects were given breaks to monitor participants' drowsiness or lack of attention. However, the active level of such listening might not be high, because except for listening to the stimuli, subjects are not required to respond or have any further action. Therefore, the active involvement level seems to be lower in the GPIAS test than the GIN test, which requires the subject to press the response button whenever they hear a gap.

Although little is known about GPIAS in higher-level cognitive processes, prior studies based on the phenomenon of SPIAS inhibition offer a prediction for the possibility of what is going to happen in GPIAS when higher-level cognition is involved. For example, when adults' attention is directed toward the pre-pulse, inhibition of the startle response is greater (Filion et al., 1993). Moreover, the anticipation of electrical shock can increase general vigilance, enhance processing

of the pre-pulse stimulus, and augment prepulse startle inhibition (Grillon, Dierker, & Merikangas, 1997).

Some authors, therefore, assumed that adding instructions for active listening for the gaps could lead to a better GPIAS measure result in distinguishing people with tinnitus from control subjects (Shadwick & Sun, 2014). Such attention-driven inhibition augmentation would technically favor healthy subjects because individuals with tinnitus might have task-based attention deficit (Araneda et al., 2015; Das, Wineland, Kallogjeri, & Piccirillo, 2012; Mannarelli et al., 2017; Mertens, Kleine Punte, De Ridder, & Van De Heyning, 2013; Tegg-Quinn, Bennett, Eikelboom, & Baguley, 2016). However, if active listening is required in the GPIAS test, attention should be controlled. Still, controlling procedures might be confounded by other factors, such as the baseline fatigue level and individual compliance, which implies that attention would have to be monitored. Therefore, passive listening might be a more practical option in GPIAS test settings.

Table 5 presents comparisons of GPIAS human studies. What we can see is different studies used different parameters in their tests. Results can be positive or negative, but all concluded that it is not because of tinnitus filling in the gap.

Of note, Ku used 20, 50, and 100 ms gaps in the tests, but only found a lack of startle inhibition with the 20ms gap in the group with tinnitus. However, as a contrast, Fourniers found a lack of startle inhibition with a 50 ms gap in the tinnitus group. Top-down attention-related modulation is surmised by Ku in his GPIAS study. Ku stated that a possible explanation is that the relatively shorter duration of the gap allows less chance for top-down involvement to be effective because attention may compensate for the lack of startle inhibition with the 50 and 100ms gap. However, this theory cannot explain Fournier's results since Fournier found a lack of startle inhibition with the 50 ms gap.

### 6.3.4 Affect and Psychological Disorders in GPIAS

Startle can probe emotional processing. Fear potentiation of startle in adults is reported initially in visual studies which demonstrated that acoustic startle magnitude in adults is facilitated during the viewing of pictures with negative affective content, relative to the viewing of pictures with positive affective content (Bradley, Codispoti, & Lang, 2006; Greenwald, Cook, & Lang, 1989). Startle responses are more significant during negative pictures, smaller during positive pictures, and intermediate during neutral pictures (Waters et al., 2004). In the same study, the investigators noticed that the startle response could be rapidly facilitated by anxiety. The authors thus suggested that anxiety effect occurs as early as the pre-attentional and attentional stages, rather than at the later stage of the information processing.

Other studies have used the startle reflex as a potential marker for psychological disorders. For example, Grillon et al. ( 1997 ) found that children and adolescents whose parents had anxiety disorders showed more significant magnitude startle responses to white-noise bursts than did control participants (Grillon et al., 1997). This study also revealed that children and adolescents whose parents had a history of alcoholism showed less habituation of the startle response over trials and showed less pre-pulse inhibition. More relatedly, in a previous study that compared tinnitus complainers and tinnitus non-complainers with controls, the habituation deficit was found in the tinnitus complainers subgroup (Walpurger, Hebing-Lennartz, Denecke, & Pietrowsky, 2003).

**Table 5. The comparison of GPIAS human studies in tinnitus**

| *Author* | Threshold | Hyperacusis | Tinnitus type and etiology? | Electrophysiological test type? | Startle stimuli | Gaps number per trial | Gap duration | ISI | ITI | Background sound | The conclusion supports "tinnitus filling in the gap"? | Lack of gap-prepulse induced acoustic startle inhibition in the tinnitus group? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ku 2017* | 250 Hz~8KHz, no > 8KH | not reported | not reported | N1 P2 of EEG | 20 ms, 65 dB SL, 1 kHz TB | 1 | 100,50, or 20 ms | 100 ms | 1~3 s | 20 dB SL, 600 Hz or 8KHz continuous PT noise | no | yes |
| *Shadwick 2014* | 250 Hz~8KHz, no > 8KHz | not reported | not reported | ocular EMG | 50 ms, 100 dB SPL (or vary individually if the subject has HL), BBN | 1 | 100 ms | not reported | 20~30 s | 38~40 dB SPL, 100 Hz bandwidth centered at the frequency of patient's tinnitus, NBN | no | no |
| *Fournier 2013* | 250 Hz~16KHz | yes, tinnitus group tend to have hyperacusis (P<0.005) | chronic high pitch tinnitus (sound like ringing only), bilateral and continuous. | ocular EMG | 50 ms, 105 dB(A) SPL, BBN | 1 | 50 ms | 120 ms | 15~23 s | 65 dB(A) SPL, centered at 500HZ (200-1.2 kHz), or 4KHz (3.5-4.5KHz), continuous NBN noise | no | yes |

Gap-startle interval: the interval between the offset of a temporal gap and the onset of the intense sound stimulus). ITI: Inter-trial interval (i.e., the intervals between the trials). EEG: electroencephalogram. EMG: Electromyography. TB: Tone burst. BBN: broadband noise. NBN: narrow-band noise. PT: pure tone. SPL: sound pressure level. GPIAS: gap-prepulse inhibition of the acoustic startle

**Table 6. The comparison of GIN human studies in tinnitus**

| *Author* | Threshold | Hyperacusis | Tinnitus type and etiology? | Electrophysiological test type? | Startle stimuli | Gaps number per trial | Gap duration | IGI | ITI | Background sound | The conclusion supports "tinnitus filling in the gap"? | GIN deficit in the tinnitus group? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Mahmoudian 2013* | 250 Hz~8KHz, no > 8KHz | not reported | chronic idiopathic tinnitus, either unilateral or bilateral tinnitus | MMN of EEG | Three stimuli paradigm, not GPIAS and not standard GIN test either | 1 | 7 ms | (the only one gap is placed in the middle of the tone) | 500ms | 75 standard stimuli 65dB SPL (0.5, 1 and 1.5 kHz); 25 ms deviant stimuli. | no | yes |
| *Boyen 2015* | 250 Hz~8KHz, no > 8KHz | not reported | bilateral continuous tinnitus, no other info | no | N//A | 12 | <30 ms | 100-200 ms | no info | 5, 10, 25 dB SL. 4 kinds of NBN: 4k - 8kHz with 1-octave noise, 4k -5 kHz with 1/3 octave noise, 5 -6.3 kHz with 1/3 octave noise, and 6.3 K -8KHz with 1/3 octave noise. | no | no |
| *Campolo 2013* | 250 Hz~16KHz | not reported | chronic continuous tinnitus, both unilateral and bilateral; buzzing/ringing/hissing/pulsing | no | N/A | 18 | 50 ms | randomly spaced | no info | 90 s, 1/3 octave wide NBN centered at 1KHz ~ 16KHz | no | no |
| *Gilani 2013* | 250 Hz~8KHz, no > 8KHz | not reported | chronic continuous tinnitus, both unilateral and bilateral tinnitus, no other info | no | standard GIN test, and gap duration pattern test | 0~3 | 2~20 ms in GIN, 200/500 ms in DPT | no info | no info | 6 s, white noise at 50dB SL | no | yes |

IGI: Inter-gap interval (i.e., the interval between the two gaps). ITI: Inter-trial interval (i.e., the intervals between the trials). MMN: mismatch negativity. EMG: Electromyography. TB: Tone burst. BBN: broadband noise. NBN: narrow-band noise. PT: pure tone. SPL: sound pressure level. GIN: gaps in noise.

### 6.3.5 Habituation in GPIAS

Other factors are closely related to GPIAS results. Schilling et al. (2017) gave rats 200 trials with gaps and another 200 trials without gaps. It showed that rats almost fully habituated to the startle stimuli after 200 trials of startle (Figure 16).

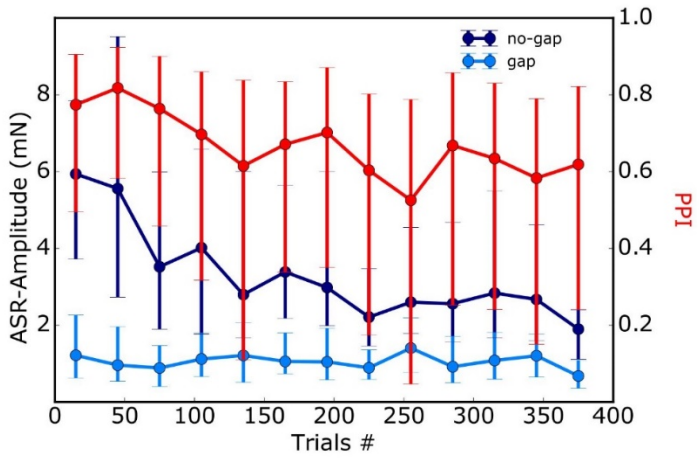



**Figure 16. Habituation effects during startle measurements. ASR amplitudes to startle pulses with and without pre-stimulus (cyan, blue, median ± (2 kHz, 200 trails gap and no-gap condition each, cf. sec. 3.1) as a function of the ASR trials. The data are binned (30 trials per bin). The fractional ASR amplitude decrease is not constant (i.e., not the same slope) for ASR amplitudes during the gap and no-gap conditions, and hence the PPI (i.e., SPIAS) values decreased as a function of ASR trials. The value mN of the coherent amplitude, for which the squeezing parameter is minimized, versus N. (with permission from Schilling et al. 2017 )**

The no-gap trials habituated much slower than the gap trials in Schilling's study. This phenomenon could be explained in part by a much lower acoustic startle amplitude of gap trials, which might blur the habituation to be observed in gap trials. In addition to the data from rats, we

also have the habituation study on SPIAS paradigms in humans. In human studies of age effect on habituation, prepulse inhibition demonstrated an inverted U-shaped function with age (greatest SPIAS at intermediate ages). At the same time, there is no significant effect of age on startle habituation magnitude. The study from Ellwanger et al. (2003) included 97 nonclinical subjects with the most extensive age range (19.6 – 83.1 yrs) classified into four groups with mean ages of 21.5, 29.1, 41.3, and 74.3, respectively. Younger age is associated with a larger startle amplitude, but age is not associated with the magnitude of habituation. In this study, there were 94 trials in four blocks in total, following the order of block 1 (5 pulse-only trials), block 2 (12 pulse-only trials + 24 SPIAS trials+ 6 no-stimuli trials), block 3 (the same as block 2), and block 4 (the same as block1) (Ellwanger, Geyer, & Braff, 2003). Although it is not clarified in the original article concerning the habituation speed across age, based on the plot, the startle response is almost fully habituated after two blocks across all age groups. Among the first two blocks, 41 startle stimuli are presented. The old age group habituated faster according to the plotted figure since the initial startle magnitude is the lowest in this group compared to the other three younger groups (Figure 17). All mammals have startle responses (Varty, Braff, & Geyer, 1999). There is no evidence showing that humans are more or less likely to startle than other mammals, but humans seem to habituate much faster than rats (Abel, Waikar, Pedro, Hemsley, & Geyer, 1998; Ellwanger et al., 2003; Schilling et al., 2017). Long-term habituation of startle in rodents is observed after several days of experiments (Figure 18) (Valsamis & Schmid, 2011).

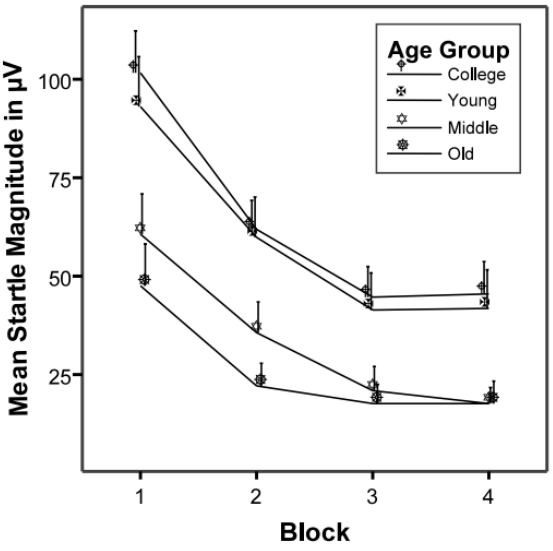



**Figure 17. Mean pulse-alone startle magnitude measured in microvolts as a function of age and block. Vertical lines show S.E. of the means. Habituation is illustrated in the magnitude decrement across blocks. (With permission from Ellwanger et al. 2003)**

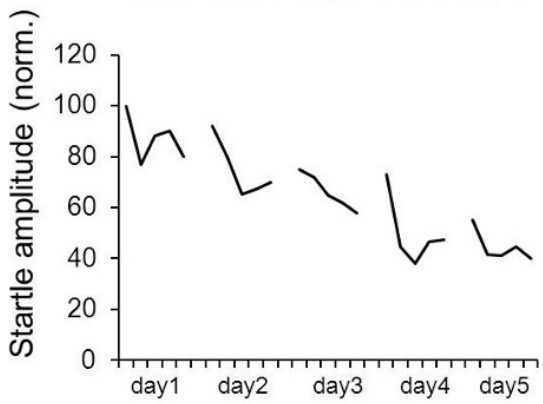



**Figure 18. Normalized startle amplitudes of 18 mice over five days. (With permission from Valsamis & Schmid 2011).**

A study from Abel et al. (1998) provided repeated testing of prepulse inhibition and habituation of the startle reflex in healthy human controls. Data are based on 23 subjects (11 females and 12 males), and after six trials of startle sound exposures (block 1), subjects reached

full short-term habituation. Subjects showed a significant decrement in the magnitude of startle from block 1 to block 2 but without further reduction in block 3 (Figure 19) (Abel et al., 1998). This phenomenon is called short-term habituation because such a phenomenon can be repeated after every 2 hours of wash-out period within one day, with slight but continuous decrements in the entire startle magnitudes from the first session of three blocks of experiments to the third session of the same three blocks of experiments. In most cases, absolute startle amplitudes differ considerably among animals, and startle magnitudes are not normally distributed. Thus, Valsamis and Schimid (2011) suggested normalizing the data of each animal or human subject to its first startle, or the average of the first two startle trials in the beginning block. However, other than the reason for normalizing data, if the study is not focused on the analysis of the habituation effect, there is a lack of explanation of why several trials of habituation are necessary. There is no difference in reflex habituation between sessions such that habituation showed temporal stability in an individual across the day (Figure 20). No long-term habituation data of human subjects are found in existent publications.

Schilling et al. (2017) gave five startle stimuli before the beginning of each measurement to rule out strong habituation effects based on studies from Turner et al. (2006) and Valsamis & Schmid (2011). However, an in-depth analysis of mid-term habituation effects on 200 trials with and 200 trials without a gap in a background sound revealed a mid-term continuous habituation effect in gerbils (Figure 16). This matches with findings from Abel and Blumenthal (2017), in which the authors found slight, continuous, but non-statistical significant differences across three sessions with one day (Figure 20). A total of three sessions included 36*3=108 trials. Between those sessions, there is about 2 hours rest period for each subject. All three sessions are completed within one day. Turner et al. (2006), and Valsamis and Schmid (2011) completed their experiments

based on around 30 trials. It could be that 30 trials are not a big sample size, and no habituation effect is found statistically within the first 30 trials. Still, once being extended to over hundreds of trials, then a mid-term habituation effect could be found. In humans and animals, to avoid mid-term habituation effects to be involved in the analysis, hundreds of trials can be administered in several separate sessions across a day with a certain amount of time between sessions (Abel et al., 1998). Alternatively, the data across all trials can be binned together and averaged for analysis (Schilling et al., 2017).

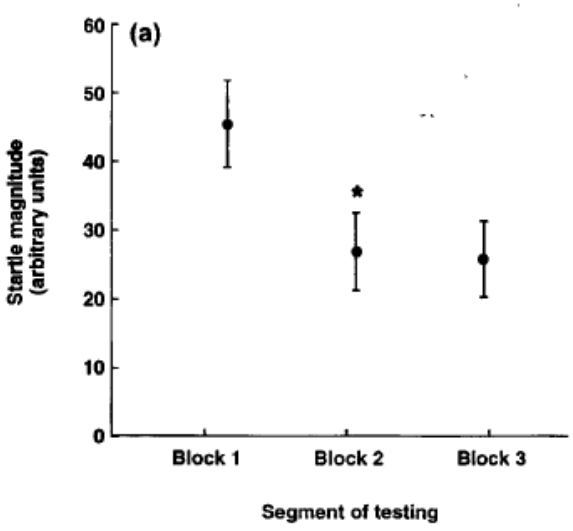



**Figure 19. Mean pulse-alone eye-blink startle magnitudes (measured in arbitrary digitalized units) (n =15). Subjects showed a significant decrement m magnitude from Block I to Block 2 (habituation), with no further reduction m Block 3 (Abel et al., 1998).**

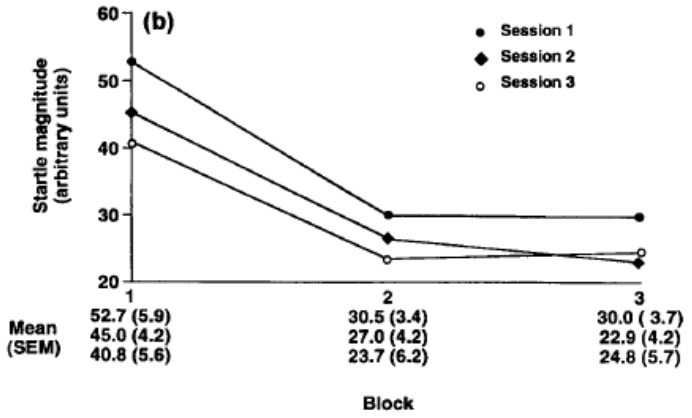



**Figure 20. Eye-blink startle magnitudes (startle-only; mean) measured with arbitrary digitalized units at Blocks 1, 2, and 3 m all subjects. There is no difference in reflex habituation between females and males or between sessions (see text) such that habituation showed temporal stability in an individual across the day (with permission from Abel et al. 1998).**

To summarize, short term habituation effect can be reached when giving rats or human subjects 5~6 trials of startle sound before the short period (e.g., no more than 30 trials) of GPIAS or SPIAS experiments start. If more trials are needed based on the study design, then a long enough washout period is required between sessions. Otherwise, due to the mid-term habituation effect, data have to be binned and averaged for analysis. For studies that require repeated measures of startle across several days, long term habituation effects have to be considered in the data analysis as well. Age and gender are also factors that affect habituation; therefore, age and gender are better to be balanced in the study design.

### 6.3.6 Other Factors in GPIAS

Gender differences are factors that should be considered during study design or at least during statistical analysis since males show a more robust prepulse inhibition than women in general (Ornitz, Guthrie, Sadeghpour, & Sugiyama, 1991). Startle variability resulting from hormone fluctuations of the estrous cycle has been found in female mice and human subjects (Plappert et al. 2005; Ison & Allen, 2007; Kumari et al., 2004). In another study of fear-potentiated startle, gender differences are found in the startle responses of adolescents whose parents had a history of anxiety disorders.

Regarding the question of age effect, behavioral evidence of gap detection in older adults suggests that they could identify the gap as precisely as younger adults when gap durations were longer than 9 ms (Harris, Wilson, Eckert, & Dubno, 2012).

Hearing loss and hyperacusis need to be controlled carefully. Taking hearing loss as an example, in Campolo's study, their subjects with tinnitus had various hearing thresholds from normal to profound hearing loss, and several subjects had very high (poor) thresholds at some frequencies. Therefore, NBN gap stimuli could not be presented at 15 dB SL for all of the 13 subjects, both ears and across all targeted frequencies. Such data would bring in potential confounds for the interpretation of results. Studies should not include subjects with moderate and severe hearing loss to avoid such issues. Moreover, for better comparative purposes, studies should include subjects with matched hearing threshold levels between groups.

The ocular electromyogram (EMG) is widely accepted as the gold standard measure of the human startle eyeblink response (Lovelace, Elmore, & Filion, 2006). Fournier (2013) and Shadwick (2014) used ocular EMG to measure startle, which is a widely used startle measurement (Shadwick & Sun, 2014). Still, there are a few differences between the studies from Fournier

(2013) and Shadwick (2014). Fournier completed hearing threshold measurement, including 250 Hz to 16 kHz, and only included subjects who had a normal audiogram. At the same time, Shadwick did not measure the threshold between 8 kHz and 16 kHz and included subjects with hearing loss (Shadwick & Sun, 2014). In terms of hearing threshold level control, Fournier's study is designed most ideally. Moderate to severe hearing loss may affect the perception of the startle stimuli in the test. For example, in Shadwick's study, they used 100 dB SPL BBN for those who had a normal or near-normal hearing but had to adjust the intensity level individually according to those subjects who had moderate-to-severe hearing loss. However, it is less clear what the role of extra-high frequencies play in GPIAS. The presence of tinnitus identified by the GPIAS method was initially supported by some animal studies (Turner, Larsen, Hughes, Moechars, & Shore, 2012; Yang et al., 2007). In the study by Yang et al. (2007), salicylate injected rats were measured with the GPIAS paradigm with an accurate threshold measure. The GPIAS paradigm revealed a lack of startle inhibition at 16 kHz, but only a significant temporal threshold shift at 12 kHz, which did not show a startle inhibition deficit. Such inconsistent results indicated that the temporal threshold shift is not related to a startle inhibition or potential presence of tinnitus. Although Liberman's tinnitus study in human subjects for the first time presented the hypothesis that extra-high frequency hidden hearing loss might be the reason that led to tinnitus, the following human tinnitus studies from Guest (2017), Paul (2017) and others did not find any extra-high frequency difference between subjects with and without tinnitus (Guest, Munro, Prendergast, Howe, & Plack, 2017; Paul, Bruce, & Roberts, 2017). Therefore, tinnitus is less likely or, at least, not merely due to the extra-high frequency hearing threshold shift. Nevertheless, it is still good to control this factor since existent evidence is either for or against the positive relationship between the extra-high frequencies and tinnitus.

Regarding hyperacusis, Fournier's study is the only one that provided hyperacusis information and also excluded subjects with hyperacusis among the seven human studies. Hyperacusis might indirectly affect the magnitude of startle response through the aversive emotional reaction that subjects may have; thus, it is reasonable to be considered into the exclusion criterion (Tyler et al., 2014; Vaidyanathan, Patrick, & Bernat, 2009). Nevertheless, hyperacusis should likely not impact startle inhibition since the startle inhibition is calculated using startle magnitude as both the numerator and the denominator and, therefore, is essentially a relative comparison on startle magnitudes.

An appropriate startle stimuli intensity is needed (Longenecker & Galazyuk, 2012). In the study from Longenecker and Galazyuk (2012), startle input-output function is a sigmoid shape; thus, changes in gap detection deficits are hardly detectable if the startle stimulus is at a high-intensity level, such as 120 dB SPL. When the startle stimuli intensity dropped to 90 dB SPL, there is a susceptible startle magnitude change.

An appropriate startle stimulus type, along with the appropriate duration of startle stimuli, also can affect results. A typical sudden, brief loud burst of wide-band noise (WBN) is the most reliable and effective eliciting startle stimulus compared to narrowband noise (NBN) (Blumenthal & Berg, 1986). The duration of startle stimuli does not show a significant gap induced startle inhibition difference if between 20~50 ms. However, the inhibition difference is significantly different between durations shorter than 50 ms and longer than 50 ms. Startle stimuli fall within the range of 20~50 ms in all the human GPIAS studies (Table 5).

Lastly, other factors, such as binaural or monaural, chronic or acute, continuous or pulsatile, tonal or non-tonal tinnitus, might also affect either GPIAS or GIN results to a different extent. However, no study has been published regarding these factors. Across GPIAS and GIN

human studies, Fournier specified that they only included bilateral, chronic, high pitch, ringing sound like tinnitus. In contrast, Mahmoudian, Boyen, Campolo, Gilani included both unilateral and bilateral tinnitus with or without reported tinnitus types. At the same time, Shadwick and Ku did not report this information at all. Looking into all seven human studies (GPIAS & GIN) from multiple angles (Table 3-5), Fournier's study has the highest quality control, followed right after by studies from Ku and Mahmoudian. The quality control level of studies from Campolo and Gilani is a little worse than the above three studies but still better than studies from Shadwick and Boyen. The ideal parameters of the factors, as mentioned above for GPIAS, are listed below in Table 7.

**Table 7. Ideal parameters of several factors in GPIAS**

| Factors | Ideal parameters |
|---|---|
| electrophysiological measurement of startle | Ocular EMG |
| Gap duration | 20 ms~100ms. Unclear, but longer duration leads to more substantial inhibition |
| Pitch match | Pitch matching with background sound pitch in each individual |
| Loudness match | Loudness matching with background sound loudness in each individual |
| Startle stimuli type and intensity | White noise burst, 90~105 dB SPL [measured by sound level meter], with instant rise time, for subjects without hearing loss; The startle stimuli might need to vary if subjects have hearing loss. |
| Duration of Startle Stimuli | 20~50 ms |
| Attention | Passive listening |
| Psychologic/neurologic diseases | Exclude |
| | |

| **Table 7 (continued)** | |
|---|---|
| Habituation effect | For short-term habituation, several startle-only trials are often applied to avoid a strong habituation effect. |
| ITI | ITI should be randomized between 10 s and 30 s, or a stable value in between. ITIs below 10 sec should be avoided to prevent effects caused by muscle fatigue and refractory periods of muscle responses(Valsamis & Schmid, 2011). |
| Gap-startle interval | 0~120ms. Unclear, but shorter interval leads to more considerable inhibition |
| Gender | Balance gender distribution between groups if possible |
| Age | Not a problem unless the gap duration is <9 ms |
| Hearing threshold | Avoid moderate and severe hearing loss |
| The extra high-frequency threshold | Not a big problem, but better to be controlled, at least statistically |
| Hyperacusis | Exclude |
| Others | Binaural/monaural; tonal/non-tonal; chronic/acute; some psychological medications and psychological disorders |

ITI: inter-trial interval. SPL: sound pressure level; EMG: electromyography

## 7.0 Math in GPIAS

In either animal or human-based studies, the investigators had to determine if GPIAS is significantly different among comparison groups by the calculation of startle inhibition change. The goal of all calculation methods is the same, which is to catch the reduced startle inhibitions in subjects with tinnitus if such inhibition reductions are existent. In healthy control subjects, the gap in the ongoing background sound, which exists several milliseconds before the startle stimuli are presented, can inhibit the startle response. The hypothesis is that if there is tinnitus, then the tinnitus will be filling in the gap. Therefore, the "change" in ongoing background sound if the background sound has been matched to the individual's perceived tinnitus will not be as noticeable and will not provide the cue that leads to startle inhibition. Thus startle inhibition in subjects with tinnitus is suppressed (less inhibition) compared to the control. This control can be accomplished using (1) a no-gap condition (i.e., ongoing sound continues without a gap), (2) subjects without tinnitus, (3) subjects before noise or ototoxic drug exposure, or can be (4) a certain pre-defined threshold for GPIAS ratio or (5) tinnitus index. This section reviews these five calculations of startle inhibition change that have been employed in previous studies.

## 8.0 Comparison of Inhibition Percentage

### 8.1 Comparison of Inhibition Percentage with Within-Subject Design

A statistically significant lack of startle inhibition in GPIAS testing after tinnitus induction is the most commonly used criteria for identifying animals with startle inhibition deficits. Researchers calculate and compare the percentages of inhibition before and after tinnitus induction (i.e., noise or ototoxic drug exposure) based on equation #1. If the percentage of inhibition level is reduced after tinnitus induction, then the animal is assumed to be experiencing tinnitus. This percentage of inhibition reduction in GPIAS is often observed at a narrow bandwidth in salicylate and noise-induced tinnitus animal models, as well as in human subjects (Fournier & Hébert, 2013; Hu, Mei, Chen, Huang, & Wu, 2014; Koehler & Shore, 2013; Middleton et al., 2011). The percentage of inhibition for each gap condition is calculated using the following equation #1.

$$percentage\ of startle\ inhibition\ in\ gap\ condition = \frac{startle\ magnitude\ of\ gap\ condition - startle\ magnitude\ of\ startle\ only\ condition}{startle\ magnitude\ of\ startle\ only\ condition} \quad (1)$$

The disadvantage of this method is that multiple-step statistical analysis decreases the power since each step requires a specific sample size or power to show significance. Therefore, studies might fail to show any difference if they have a similar sample size to the other studies that used a one-step statistical analysis. However, this method allows subjects to self-balance several confounding factors, such as hearing threshold, gender, and age.

### 8.2 Comparison of Inhibition Percentage with Between-Subject Design

Some labs judge tinnitus based on the comparison of startle inhibition between the exposure group and the non-exposure group assuming that some of the animals in the exposed group would develop tinnitus following noise exposure (Llano et al., 2012). The gap inhibition is still calculated based on equation #1.

The advantage of comparing inhibition results between the groups is a better control of the natural aging effect in small animals such as rodents compared to the within-subject design. This method supports research that is interested in the observation of tinnitus-related neurophysiological changes over time following noise exposure or ototoxic drug administration. However, this method requires a larger sample size compared to those self-comparative methods. Also, this method needs researchers to balance the confounding factors between groups, such as hearing thresholds, sex, and gender.

### 8.3 Comparison of Startle Amplitude with Between-Subject Design

Other laboratories chose not to use the above equations, but rather directly compared the startle amplitude in no-gap (condition *a*) vs. amplitude in gap trials (condition *b*) statistically to see whether an animal developed tinnitus (Kraus et al., 2011; Mulders, Barry, & Robertson, 2014). The conditions of *a b* are referring to no-gap/gap conditions in Figure 10.

The amplitude of the acoustic startle response is measured in the noise-exposed animals. If such comparison is not significantly different in some animals, they are assigned to the tinnitus-positive group. The advantage of this method is that it considers individual differences after noise

exposure. In other words, some individual animals with the same noise exposure may not develop tinnitus. So this method allows researchers to exclude those who have less likely developed tinnitus after noise exposure/drug exposure, which benefits researchers who are trying to explore the protecting factors behind the scene that some animals do not develop tinnitus after the same amount of noise or drug exposure.

### 8.4 GPIAS Ratio Threshold and Tinnitus Index

One lab used a fixed threshold for GPIAS ratio above which animals would be considered as tinnitus positive, and another lab developed a tinnitus index (Middleton et al., 2011; Norman, Tomscha, & Wehr, 2012). The downside of these two methods is the same since both GPIAS ratio threshold and tinnitus index are established based on specific lab data, therefore whether these two methods can be generalized out of these labs remains unclear, especially when there is no replication of such calculations in other studies. Nevertheless, this method has its strength in its flexibility of applying to either within-subject design or between-subject design studies.

**Table 8. Summary of Calculation Methods in GPIAS**

| Calculation Method | Study Design | Pros | Cons |
|---|---|---|---|
| comparison of inhibition percentage | within-subject | self-balance confounding factors | decreases power |

| **Table 8 (continued)** | | | |
|---|---|---|---|
| comparison of inhibition percentage | between-subject | suitable for observations over time | need a larger sample size, and cannot self-balance confounding factors |
| comparison of startle amplitude with between-subject design | between-subject | considers individual differences after noise exposure | unclear |
| GPIAS ratio threshold and tinnitus index | within or between subject | can be applied to either within-subject designed or between subject designed studies. | generalization issue, lack of study replication |

## 8.5 The Summary

The literature review of GPIAS studies in both animals and humans indicates that the current evidence does not provide answers to the question of whether the GPIAS paradigm is exclusively testing tinnitus, and whether measured inhibition deficits are due to tinnitus filling in the gap.

A human can communicate the subjective perception of tinnitus and quantify the loudness and pitch, while animals cannot. Therefore, the validation of the GPIAS paradigm in humans is necessary and may provide the information needed to verify the animal tinnitus models, so the search for the neural mechanism of tinnitus generation can continue more confidently. However, unlike the extensive studies conducted on animals, minimal research has focused on this issue in humans. Some researchers employed GIN as a substitute for GPIAS in testing tinnitus in humans.

However, these two testing paradigms are not comparable. In human studies that adopted a GPIAS paradigm in testing tinnitus, conclusions regarding whether there is a lack of gap induced startle inhibition in subjects with tinnitus are controversial. Those who found the inhibition deficit in subjects with tinnitus concluded that such inhibition deficit is not due to the theory of tinnitus filling in the gap. Still, their findings could not either strongly support or negate what they concluded. The authors who stated that tinnitus filling in the gap is not likely the reason for their observed lack of startle inhibition in the group with tinnitus proposed two alternative explanations, including the top-down attention modulation theory and the continuity illusion theory to explain their results.

Ku used 20,50 and 100 ms gaps in their tests but only found an inhibition deficit with a 20 ms gap in subjects with tinnitus, therefore the top-down attention-related modulation theory is proposed by Ku et al. This theory suggests that a relatively shorter duration of gap (i.e., 20ms) allows less chance for top-down involvement to be effective because attention may compensate for the inhibition deficit in the GPIAS test when the gap is longer (i.e., 50 and 100ms). However, this theory cannot explain Fournier's (2013) results, which showed a lack of gap induced startle inhibition with a 50 ms gap in the group with tinnitus.

Fournier's (2013) GPIAS result could be alternatively explained by the continuity illusion theory (Figure 21) proposed by Mahmoudian et al. in their GIN study. Mahmoudian et al. found a non-frequency-dependent gap detection deficit in the tinnitus group using the mismatch negativity measure. Therefore, Mahmoudian et al. cited a study from Riecke et al. (2008) and used Riecke et al.'s continuity illusion theory to explain their results. This theory indicates a phenomenon that a sound which is briefly interrupted by a silent gap is perceived as discontinuous. Still, when the gap is filled with noise, the sound may be perceived as continuing through the noise. Based on Riecke

et al.'s finding, if the gap duration is shorter than 900 ms, people should have continuity illusion when the steady-state background tone has a frequency between 500 Hz and 6000 kHz. The continuity illusion theory could explain Fournier's results since Fournier et al. (2013) found a 50 ms gap-related startle inhibition deficit in the group with tinnitus when tested with both 500 Hz and 4 kHz background sound. In contrast, almost none of their subjects with tinnitus had 500 Hz tinnitus. Nevertheless, continuity illusion should not be sensitive over a small gap duration difference; thus, it cannot explain the results from Ku's study, which revealed a lack of gap caused startle inhibition with a 20 ms gap but not a 50 or 100 ms gap.

Therefore, none of the two alternative theories could explain all currently existent human study findings.

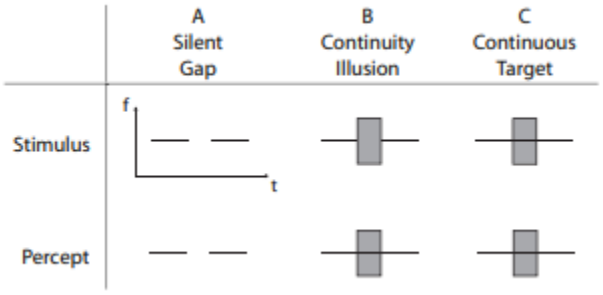



**Figure 21. The auditory continuity illusion, exemplified for steady-state tones. (A) Two tones interrupted by a silent gap are perceived as two independent entities. (B) The illusion of a single entity when a broadband noise masker is added to the gap, a percept that is like a physically continuous tone, shown in Panel C (with permission from Riecke et al. 2008).**

Interestingly, forward masking of gap (Figure 22) can be used to explain the above findings across studies. The hypothetical theory of forward-masking of the gap is defined as the disappearance of the gap in the GPIAS condition caused by the masking effect from the ongoing

background sound played right before the gap. Although it seems like this cannot explain Fournier's results, because Fournier found an inhibition deficit with a 50 ms gap rather than 20 ms, Fournier used 65 dB SPL NBN while Ku used 20 dB SL pure tone as background sound. Regardless of the different sound types, the intensity of the background sound plays a role in different GPIAS performance. Since the higher intensity of background sound lets the forward masking last longer, Fournier could find a lack of startle inhibition in the tinnitus group with a 50ms gap. In contrast, Ku could only find such an inhibition deficit with a 20 ms gap.

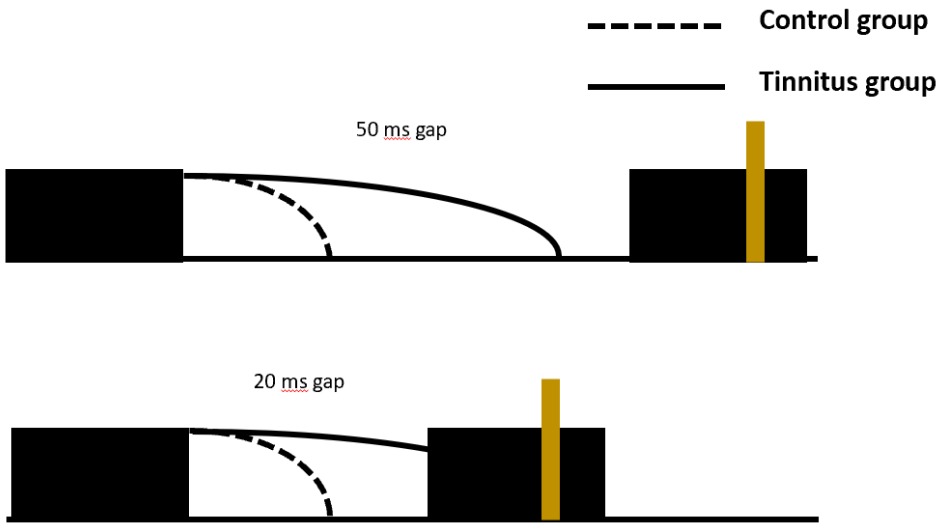



**Figure 22. Forward Masking of Gap**

To summarize, none of these human GPIAS studies yielded strong evidence to either uphold or negate the gap-filling theory that would predict the startle inhibition results. Therefore, to answer these questions, ideal parameters are summarized in Table 7, listing what should be employed or avoided in future studies. Among all those factors, an optimal match between background sound and tinnitus, considering loudness and pitch, might be weighted as the most critical parameters. Validating GPIAS in the human will facilitate answering essential questions,

which are critical for developing the neuroscientific framework of tinnitus generation and treatments targeting those generation elements. Answers to these questions may alter or support the current preponderant dependence of the GPIAS paradigm in studies of tinnitus in animals.

## 9.0 A Gap or A Different Signal

The theory of forward-masking of gap and Ku's study results indicated that certain levels of change to the gap duration would result in a startle inhibition change. Additionally, several SPIAS studies revealed that specific changes in sound prepulse could lead to a startle inhibition change. For instance, Longenecker et al.'s (2016) study measured hearing threshold in mice using ABR, and they found that mice with hearing loss after sound exposure had fewer inhibitions of SPIAS compared to the inhibitions before sound exposure. Since the study used SPL instead of SL for acoustic startle, both the acoustic prepulse and acoustic startle would sound weaker to those mice that had hearing loss after sound exposure compared to before the exposure of sound. In other words, the intensity change of a sound prepulse could result in a change of startle inhibition.

Studies from Blumenthal (1995, 1996) and Swerdlow et al. (2007) further confirmed that the inhibition of startle increases with both prepulse intensity and duration. In Swerdlow's (2017) study, the 50 ms prepulse duration seems to lead to the most significant percentage of startle inhibition, compared to 5, 20, 120 ms of prepulse duration conditions. According to Bloch's law, total luminous energy is a constant value (k), this threshold is reached when the product of luminance (L) and stimulus duration (t) equals this constant (Hildreth, 1973). When luminance is halved, a doubling in stimulus duration is required to reach the threshold and vice versa. However, when luminance L and stimulus duration t both increase, total luminous energy k also will increase. Bloch's law (a rule of temporal summation) is expressed as $\mathbf{L.\ t^n = k}$. Braff et al., (2001) believed that Blumenthal's (1995, 1996) study findings follow Bloch's law that prepulse stimulus salience reflects an interaction of intensity and duration, among which the stimulus salience is the same as

total luminous energy k while prepulse intensity is the luminance L and prepulse duration is the t. Of note, the original Bloch's law is valid only when the stimulus duration is no longer than 100 ms (Scharnowski, Hermens, & Herzog, 2007). Similarly, the prepulse stimulus duration should be within a short-range if Bloch's law is to be applied as an explanation.

Regarding the effect of frequency change of sound prepulse on the change of startle inhibition level, Clause et al. (2011) found a ~45% startle inhibition change for a frequency change of 3.3%, 64 ± 4% startle inhibition change for a 25% frequency change. Stitt et al. (1974) used a comparably sized transition between one octave-wide band of random noise and found a 60% startle inhibition change.

Compared to the SPIAS studies of the effect of intensity change and frequency change of sound prepulse on the change of startle inhibition, there are almost no studies except for one from Swerdlow et al. (2007), which demonstrated a significant effect of prepulse bandwidth on the startle inhibition change.

The above mentioned SPIAS evidence also points to the possibility that a silent gap and a different signal from the ongoing background sound in the gap space should both cause inhibition of the startle in GPIAS conditions. Specifically, certain degrees of partial filling of the gap can potentially lead to the same change of inhibition level as full filling of the gap. In contrast, some other degrees of gap-filling may lead to a different change of inhibition level from the full gap filling. In other words, the (partial) gap-filling sound may be varied in intensity, frequency, bandwidth, and timbre to modulate the startle response differentially. For example, a gap-filling sound can be otherwise the same as the ongoing background sound except for the difference in frequency. The frequency contrast between the ongoing background sound and the gap-filling sound could lead to a level of startle inhibition, similar to a quiet gap. Peterson and Blumenthal

(2018) recruited 45 subjects ( 20 in control, 25 in tinnitus group), and the authors filled the gap with 65 dB white noise, while the background sound was also 65 dB white noise. Such insertion did not cause inhibition of startle, demonstrating that the observed inhibition of startle responding was not due to any switching transient in the sound file.

Nevertheless, sound prepulse was a more efficient startle magnitude inhibitor than partial gaps, and a more considerable **intensity** change from the background sound resulted in more significant startle inhibition (Peterson & Blumenthal, 2018). The reason behind the more substantial startle inhibition ignited by sound prepulse than by gap prepulse might be due to a different neural circuit that is used when processing these two prepulses. This finding also leads to a possibility that some partial filling of the gap could work the same as full filling of the gap. However, so far, we do not know to what extent such minor differences in intensity would result in a startle inhibition difference between a full filling and a partial filling of the gap in the ongoing background sound.

Peterson and Blumenthal (2018) also claimed that just a **frequency** change could lead to startle inhibition similar to gap prepulse (Peterson & Blumenthal, 2018). The 65-dB passband prepulse resulted in significant inhibition, meaning that a change in stimulus frequency composition (broadband to passband) with no change in intensity was enough to activate inhibitory neural pathways that terminate on the startle center in the pons. This finding mirrors in humans a similar effect of a frequency shift on startle inhibition reported in rats (Cranney, Cohen, & Hoffman, 1985; Marsh, Hoffman, Stitt, & Schwartz, 1975). Even with these findings, we are still unsure whether more significant frequency change from background sound can result in more significant startle inhibition. Additionally, it is unclear to what extent such minor differences in

frequency would have a meaningful difference between a full filling and a partial filling of the gap.

In addition to frequency and intensity, bandwidth and timbre are also important stimulus characteristics that need to be considered since many subjects with tinnitus report a tonal component. Regarding **bandwidth**, Roberts et al. (2006) categorized tinnitus into only three types, including tonal, ringing, and hissing. The authors used bandwidth to describe the three types of tinnitus. Specifically, ringing tinnitus is described as plus or minus 5% of central frequency at 20 dB, and the hissing tinnitus is plus or minus 15% of central frequency at 20 dB (Roberts, Moffat, & Bosnyak, 2006). In a descriptive study focusing on examining the relationship between audiometric profile and tinnitus pitch, tinnitus bandwidth ranged from 0.13 to 0.44 kHz based on data from 67 people with chronic bilateral tinnitus (43 men and 24 women, age range 22 ~ 81 years). The bandwidth of tinnitus has been arbitrarily categorized into three groups, including a narrow bandwidth (0.13 – 0.25 kHz), a moderate bandwidth (0.26 – 0.33 kHz), and a wide bandwidth (0.34 – 0.44 kHz) (Sereda et al., 2011). These bandwidths of tinnitus were determined arbitrarily and, therefore, would cause the bandwidth of tinnitus deviates from the bandwidth of testing background sound in the GPIAS paradigm. Little is known that whether such bandwidth contrast between tinnitus perception and testing background sound in the GPIAS paradigm would result in a significant startle inhibition.

Timbre is defined as the quality by which two notes may be judged to be dissimilar when pitch and loudness have been equated (Sonn, 1973). This definition tells little about the nature of timbre itself, only that it is not subsumed by pitch or loudness. It remains ambiguous whether timbre should be measured on a categorical or continuous scale. The physical correlates of timbre are incompletely defined, although it is clear that they consist of more than steady-state harmonics

alone (Iverson & Krumhansl, 1993; Risset & Wessel, 1999). As to **timbre** in tinnitus, results suggested that the timbre of the corresponding tinnitus sensations resembled that of high-frequency, broad-band noise (Norena, Micheyl, Chéry-Croze, & Collet, 2002).

On the other hand, most patients with tinnitus could have a pure comparison tone match the pitch of their tinnitus to some extent, but with a substantial timbre difference between the two. They mostly judged the comparison tone being sharper than the tinnitus. However, no studies specifically examined tinnitus timbre by more than describing the existence of timbre differences in subjects with tinnitus. One intriguing question is whether the change of timbre in the gap would result in a different startle inhibition.

Based on the current literature, it seems that the silent gap can be replaced by a certain level of parameter change as compared to the ongoing background sound to produce an inhibition in a startle. That being said, certain levels of signal parameter mismatch between the GPIAS background sound and tinnitus perception will bring a startle inhibition change. In other words, the mismatch degree may be crucial. The mismatch degree in people with tinnitus can only be obtained by asking them to scale the matching level quantitatively. However, it is unknown whether self-perceived tinnitus matching level can validly represent the mismatch difference between GPIAS background sound and tinnitus perception. If self-perceived tinnitus matching rating is a valid way of revealing the exact mismatch level, it can be further considered as a moderator to adjust the startle inhibition level in each individual. The matching rating can be applied clinically as well if GPIAS can be used in the objective assessment of tinnitus in humans.

Furthermore, understanding how much mismatch of the ongoing background sound and a sound filling in the silent gap is needed to change the startle inhibition response also guides how many mismatches will produce no change to startle inhibition. This information would support

animal research related to tinnitus. It is almost impossible to match the GPIAS testing background sound with the tinnitus in an animal supposing that the animal truly has tinnitus. If we know within how many degrees of a mismatch there will be no change to the startle (i.e., conservative range), we can be more comfortable applying the GPIAS paradigm to animals with suspected tinnitus since the conservative range allows the animal researchers to run GPIAS testing in animals without the need to exactly match the background sound with the animals' tinnitus. As a result, findings from a well-designed study in humans may help move the tinnitus research in animals forward.

## 9.1 Research Questions and Specific Aims

The current study addressed three research questions. The primary research question (RQ1) was whether tinnitus filling in the gap of a GPIAS testing paradigm caused the lack of startle inhibition in individuals with tinnitus as compared to individuals without tinnitus. The second research question (RQ2) was whether deviations in intensity, frequency, or bandwidth of the ongoing background sound as compared to a background sound maximally matched to an individual's tinnitus induced startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation would not lead to startle inhibition. The third research question (RQ3) addresses whether the accuracy of the match between the ongoing background sound and the individual's tinnitus as defined by self-perception of the match predicts the gap induced startle inhibition percentage. The magnitude of startle response was measured with ocular EMG. The specific aims are listed as follows.

<u>Specific aims for RQ is to determine:</u>

Aim1: If there is inhibition in self-control condition as compared to the startle-only condition in individuals without tinnitus, when the background sound in the self-control condition and startle-only condition are the same.

Aim2: If there is lack of inhibition in the self-control condition as compared to the startle-only condition in individuals with continuous tonal tinnitus, when the background sound in the self-control condition and the startle-only condition are the same and are both maximally matched to each individual's tinnitus.

Specific Aims for RQ2 are to determine:

Aim1: If variation in intensity (See 3, 4, 5, 6 in Figure 25) of the ongoing background sound leads to startle inhibition change as compared to tinnitus matched self-control condition (See 2 in Figure 25).

Aim2: If variation in frequency (See3, 4, 5, 6 in Figure 26) of the ongoing background sound leads to startle inhibition change as compared to tinnitus matched self-control condition (See 2 in Figure 26).

Aim3: If variation in bandwidth (See Figure 27) of the ongoing background sound leads to startle inhibition change as compared to tinnitus matched self-control condition (See 2 in Figure 27).

Aim4: Within what level of variations in these parameters (i.e., intensity, frequency, and bandwidth) of the ongoing background sound as compared to the individual's tinnitus, will there be no significant change in startle inhibition.

Specific Aim for RQ3 is to determine:

Aim1: If a higher self-reported rating of tinnitus matching accuracy corresponds with less gap induced startle inhibition when these signals are used for the ongoing background sound (See Figure 28).

### 9.1.1 Difference Limen (DL)

The Aim 4 of RQ2 is to identify within what level of variations in the parameters (i.e., intensity, frequency, and bandwidth) of the ongoing background sound, will there be no change in startle inhibition percentage as compared to the startle inhibition percentage in the self-control condition (when the ongoing background sound is matched as closely as possible to the individual's tinnitus).

A subject with tinnitus may be aware of a certain level of a parameter change based on difference limen (DL). Still, there may be no change in startle inhibition magnitude when presenting an ongoing background sound with these parameters as compared to the startle inhibition measured when the ongoing background sound was matched as closely as possible to the individual's tinnitus (self-control condition). In this situation, we know that certain DL of parameter change in background sound would not induce startle inhibition. In contrast, if a given level of change on a parameter is smaller than the difference limen, and there is also no change in startle inhibition magnitude; it would be less informative to us whether this result is due to the reason mentioned above, or due to the subject not being aware of a parameter change because the level of change is too small. Therefore, the parameter levels for each subject must be manipulated based on his/her individualized difference limen (DL), which is the smallest level of intensity,

frequency, or bandwidth difference between two sounds that an individual subject with tinnitus can differentiate in the current study.

#### 9.1.1.1 Intensity DL

In a study focusing on the intensity and frequency difference limen (DL) using an ongoing train of 1 kHz tone burst stimuli at 60 dB SPL on both infants and young adults with normal hearing, researchers found 1-2 dB SPL intensity DL (Sinnott & Aslin, 2005). In another study using both pulsed tone and amplitude-modulated stimuli (modulation rates of 2, 4, and 8 Hz) rather than tone burst stimuli, they found about 1-2 dB SL intensity DL at 2, 4, 6, 8 and 10 kHz for lower and higher intensity stimuli (i.e., 15 to 60 dB SL) (Long & Cullen, 2005).

In a study from Carlyon & Moore (1984) using pure tones as stimuli, the intensity discrimination was measured at intensities of 35~80 dB SPL for frequencies of 500, 6500, and 8000 Hz, and at the intensity of 55 dB SL for durations of 5 ~70ms. The mean absolute thresholds of the ability to identify an intensity difference between two sounds for the 30-ms signal at 500, 4000, and 6500 Hz are 18, 9, and 14 dB SPL, respectively. Their findings are summarized in Table 9 and Table 10 (Carlyon & Moore, 1984).

Similar intensity discrimination also is measured across several stimuli intensity levels and frequencies in the study from Jesteadt et al. (2005). At stimuli of 5, 10, 20, 40, and 80 dB SL across frequencies of 400, 600, 800, 1000, 2000, 4000, and 8000 Hz, the intensity DLs are listed in the following Table 11 (Jesteadt, Wier, & Green, 2005). The intensity DL data from Carlyon & Moore (1984) and Jesteadt et al. (2005) are mostly matched.

**Table 9. Intensity DL of pure tone across frequency and intensity**

| Frequency of stimuli (Hz) | Stimuli type | Stimuli intensity (dB SL) | Intensity DL (dB SL) |
|---|---|---|---|
| 500 | Pure tone | 25~60 | ≈ 1 |
| 6500 | Pure tone | 25~30 | ≈ 1 |
| | | 35 | ≈ 2 |
| | | 40~55 | ≈ 3 |
| | | > 60 | < 1 |
| 8000 | Pure tone | 25~25 | ≈ 1 |
| | | 30~45 | ≈ 2 |
| | | > 50 | < 1 |

**Table 10. Intensity DL of pure tone across frequency and durations**

| Frequency of stimuli (Hz) | Stimuli type and intensity | Stimuli duration (ms) | Intensity DL (dB SL) |
|---|---|---|---|
| 500 | Pure tone, 55 dB SPL | 5~70 | ≈ 1 |
| 4000 | Pure tone, 55 dB SPL | 5~70 | ≈ 1 |
| 6500 | Pure tone, 55 dB SPL | 5~70 | ≈ 1 |

**Table 11. Mean values of intensity DLs across subjects and replications (Derived from Jesteadt et 2005).**

| | ***SL*** | | | | |
|---|---|---|---|---|---|
| *Frequency (Hz)* | ***5*** | ***10*** | ***20*** | ***40*** | ***80*** |
| *200* | 1.51 | 1.39 | 1.07 | 0.93 | … |
| *400* | 1.53 | 1.51 | 1.23 | 1.05 | 0.58 |
| *600* | 1.54 | 1.35 | 1.40 | 0.89 | 0.58 |
| *800* | 1.79 | 1.28 | 1.26 | 0.93 | 0.46 |

| Table 11 (continued) | | | | | |
|---|---|---|---|---|---|
| *1000* | 1.81 | 1.64 | 1.37 | 1.00 | 0.43 |
| *2000* | 1.55 | 1.33 | 1.45 | 1.17 | 0.47 |
| *4000* | 1.66 | 1.02 | 1.06 | 1.01 | 0.52 |
| *8000* | 2.78 | 1.61 | 1.19 | 1.57 | 0.56 |

### 9.1.1.2 Frequency DL

DLs for frequency as the proportion of central frequency was measured in two studies (Moore & Ernst, 2012; Vinay & Moore, 2011) (Figure 23). The study results are summarized in Table 12.

**Table 12. Mean values of frequency DLs across frequencies and subjects.**

| Central frequency in Hz | Frequency DLs as a proportion of central frequency |
|---|---|
| 0.25K | 0.02 |
| 0.5K | 0.02 |
| 1K | 0.02 |
| 2K | 0.008~0.02 |
| 4K | 0.01~0.02 |
| 6K | 0.014~0.02 |
| 8K | 0.02 |
| >8K | 0.02 |

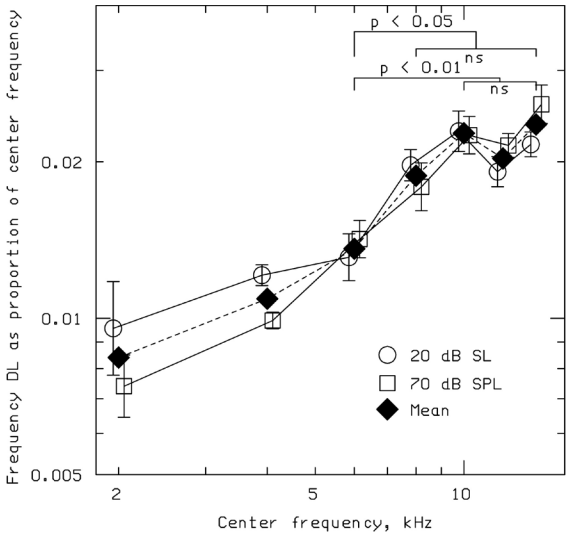



**Figure 23. Geometric mean frequency DLs across nine subjects plotted as a proportion of center frequency. Results are shown for mean levels of 20 dB SL (open circles), 70 dB SPL (open squares), and the mean of the two (filled squares). Error bars indicate ±1 standard error of the normalized frequency DLs (see text) (with permission from Moore & Ernst, 2012)**

### 9.1.1.3 Bandwidth DL

In the study from Vickers & Faulkner (1997), all subjects showed an ability to discriminate the stimuli presented to them to some extent. For the healthy hearing group, the mean 75% correct threshold bandwidth ratios were 1.30 and 1.50 for conditions 1 and 2, respectively. The DL of bandwidth (i.e., the threshold bandwidth ratio) of 1.30 for condition 1 corresponds to being able to discriminate bandwidths of 385 Hz and 500 Hz. The DL of the bandwidth of 1.50 in condition 2 corresponds to the bandwidths of 124 Hz and 186 Hz being discriminable (Vickers & Faulkner, 1997).

The results of this study are consistent with those of Pickett, Daly, and Brand (1965). They measured just-noticeable differences for the cut-off frequency of low pass noise at a variety of cut-off frequencies (250, 500, 1000, 1500, and 2000 Hz) in normal and severely cochlear damaged

subjects. If transferring their result into DL of bandwidth (i.e., the threshold bandwidth ratio), they found that at 250 Hz, the DL of bandwidth in normal hearing subjects was 1.12; representing a 30 Hz difference. At the frequencies higher than 250 Hz, their DL of bandwidth ranged from 1.03 to 1.05 (Pickett, Daly, & Brand, 2005). Thus, in general, the DL of bandwidth at higher frequencies is smaller than at lower frequencies (Table 13).

**Table 13. Bandwidth DLs as reflected by the ratio of two bandwidths**

| Study source | Frequency | Bandwidth DLs as indicated by the ratio of two bandwidths |
|---|---|---|
| **Pickett, Daly, & Brand, 2005** | < = 250 Hz | 1.12 |
| | > 250 Hz | 1.03~1.05 |
| **Vickers & Faulkner, 1997** | < = 250 Hz | 1.5 |
| | > 250 Hz | 1.3 |

## 9.2 Hypothesis

### 9.2.1 Hypothesis for Aim 1 of RQ 1

H0: Startle response in self-control condition (where there was a gap) would NOT be inhibited as compared the startle-only condition (where there was no gap) in individuals without tinnitus.

H1: Startle response in self-control condition (where there was a gap) would be inhibited as compared the startle-only condition (where there was no gap) in individuals without tinnitus.

### 9.2.2 Hypothesis for Aim 2 of RQ 1

H0: Startle response in self-control condition (where there was a gap) would NOT be inhibited as compared the startle-only condition (where there was no gap) in individuals with continuous tonal tinnitus.

H1: Startle response in self-control condition (where there was a gap) would be inhibited as compared the startle-only condition (where there was no gap) in individuals with continuous tonal tinnitus.

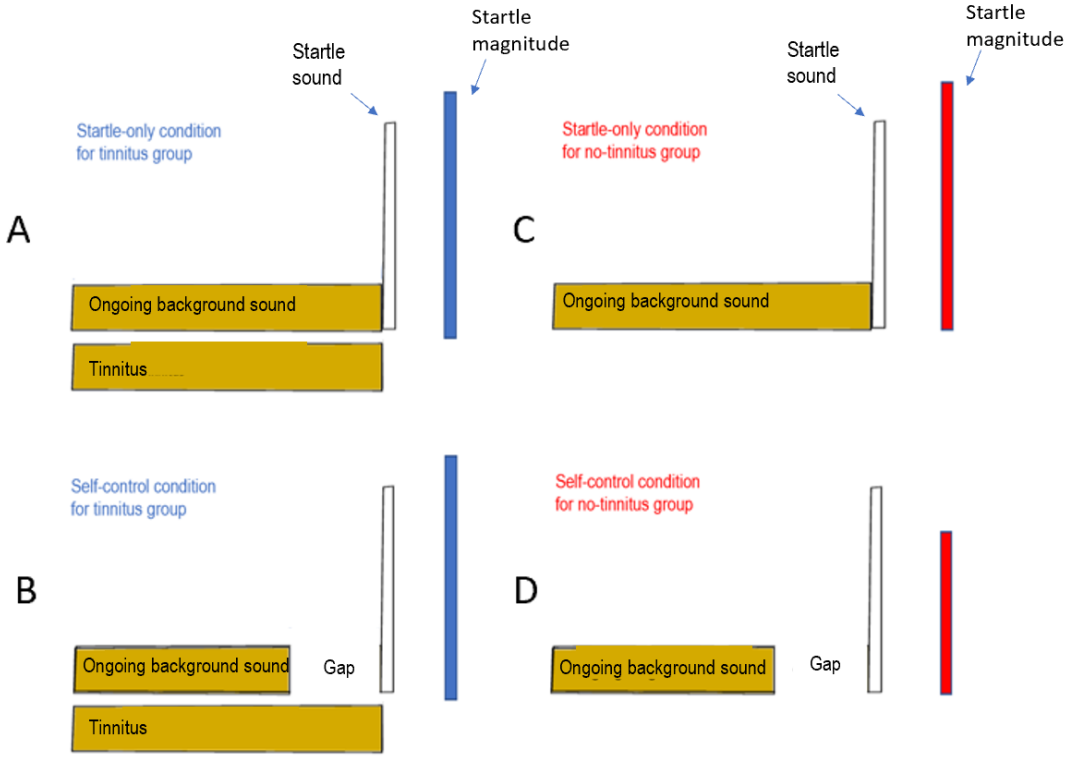



**Figure 24. Examples of the startle-only conditions and self-control condition for RQ1. The blue bar indicates the startle response from individuals with tinnitus, while the red bar indicates the startle response from individuals without tinnitus. The taller bar represents a larger response indicating a lack of startle inhibition, while the shorter bar stands for a smaller response consistent with startle inhibition. As is shown**

**in this illustration, the startle magnitude in the self-control condition was inhibited (panel D) as compared to the startle-only condition (panel C) in subjects without tinnitus (hypothetical results based on H1 of aim 1), because there was no tinnitus to fill in the gap in the startle-only condition. The startle magnitude in the self-control condition was not inhibited (panel B) as compared to the startle-only condition (panel A) in subjects with tinnitus (hypothetical results based on H0 of aim 2), because tinnitus filled in the gap in the startle-only condition, and therefore no startle inhibition was measured.**

### 9.2.3 Hypothesis for Aim 1 of RQ 2

H0: Variation in intensity of the ongoing background sound would NOT lead to startle inhibition change as compared to the inhibition percentage from the self-control condition.

H1: Variation in intensity of the ongoing background sound would lead to startle inhibition change, and a larger magnitude of variations in intensity would lead to a larger inhibition change as compared to the inhibition percentage from the self-control condition (when the ongoing background sound is matched as closely as possible to the individual's tinnitus).

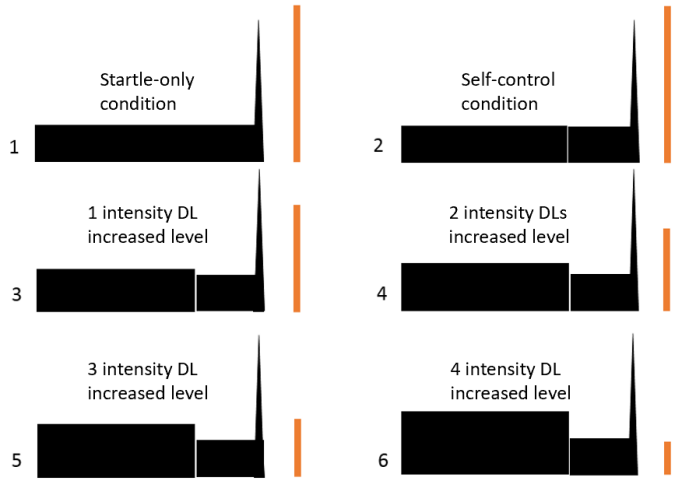



**Figure 25. The intensity-varied stimulus conditions for RQ2. The taller orange bar indicates the larger startle response, while the shorter bars indicate smaller responses corresponding to startle inhibition (hypothetical results based on H1). Startle stimulus is white noise at 100 dB SPL. The silent gap is 120 ms long. The background sound in (1) and (2) is maximally matched to the subject's tinnitus perception. The background sound is changed in its intensity from (3) to (6). Explanations: (1) background sound+ subject's own tinnitus perception+ startle sound , (2) background sound+ gap+ subject's own tinnitus pereption+ startle sound , (3) 1 intensity DL increased background sound+ gap + subject's own tinnitus perception+ startle sound, (4) 2 intensity DLs increased background sound+ subject's own tinnitus perception+ startle sound, (5) 3 intensity DLs increased background sound+ subject's own tinnitus perception + startle sound, (6) 4 intensity DLs increased background sound+ subject's own tinnitus perception+ startle sound.**

### 9.2.4 Hypothesis for Aim 2 of RQ 2

H0: Variation in the frequency of the ongoing background sound would NOT lead to startle inhibition change as compared to the inhibition percentage from the self-control condition.

H1: Variation in frequency of the ongoing background sound would lead to startle inhibition change, and a larger magnitude of variations in frequency would lead to a larger inhibition change as compared to the inhibition percentage from the self-control condition (when the ongoing background sound is matched as closely as possible to the individual's tinnitus).

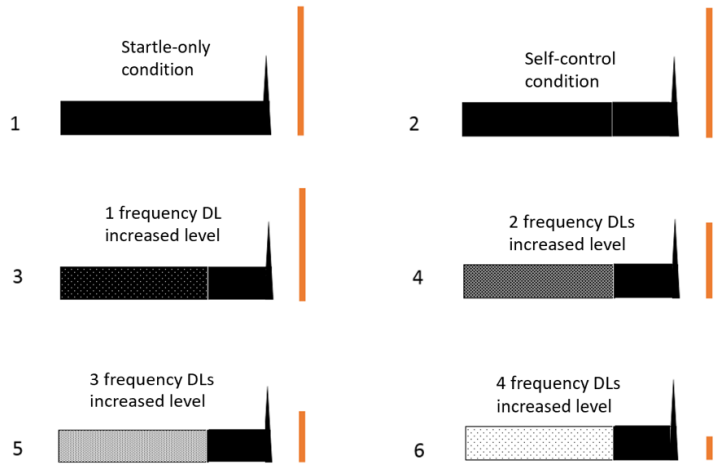



**Figure 26. The frequency-varied stimulus conditions for R2. The taller orange bar indicates the larger startle response, while the shorter bars indicate smaller responses corresponding to startle inhibition (hypothetical results based on H1). Startle stimulus is white noise at 100 dB SPL. The silent gap is 120 ms long. The background sound in (1) and (2) is maximally matched to the subject's tinnitus perception. The background sound is changed in its frequency from (3) to (6). Explanations: (1) background sound+ subject's own tinnitus perception+ startle sound , (2) background sound+ gap+ subject's own tinnitus perception + startle sound , (3) 1 frequency DL increased background sound+ gap+ subject's own tinnitus perception + startle sound , (4) 2 frequency DLs increased background sound+ gap+ subject's own tinnitus perception+ startle sound , (5) 3 frequency DLs increased background sound+ gap+ subject's own tinnitus perception+ startle sound , (6) 4 frequency DLs increased background sound+ gap+ subject's own tinnitus perception + startle sound.**

### 9.2.5 Hypothesis for Aim 3 of RQ 2

H0: Variation in the bandwidth of the ongoing background sound would NOT lead to startle inhibition change as compared to the inhibition percentage from the self-control condition.

H1: Variation in the bandwidth of the ongoing background sound would lead to startle inhibition change, and a larger magnitude of variations in bandwidth would lead to a larger inhibition change as compared to the inhibition percentage from the self-control condition (when the ongoing background sound is matched as closely as possible to the individual's tinnitus).

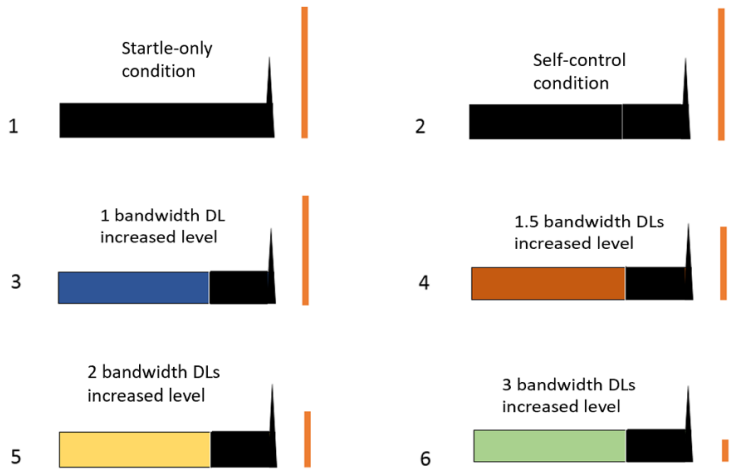



**Figure 27. The bandwidth-varied stimulus conditions for RQ2. The taller orange bar indicates the larger startle response, while the shorter bars indicate smaller responses corresponding to startle inhibition (hypothetical results based on H1). Startle stimulus is white noise at 90 dB SPL. The silent gap is 120 ms long. The background sound in (1) and (2) is maximally matched to the subject's tinnitus perception. The background sound is changed in its bandwidth from (3) to (6). Explanations: (1) background sound+ subject's own tinnitus perception+ startle sound, (2) background sound+ gap+ subject's own tinnitus perception+ startle sound , (3) 1 bandwidth DL increased background sound+ gap+ subject's own tinnitus perception+ startle sound , (4) 1.5 bandwidth DLs increased background sound+ gap+ subject's own tinnitus**

**perception + startle sound, (5) 2 bandwidth DLs increased background sound+ gap+ subject's own tinnitus perception+ startle sound, (6) 3 bandwidth DLs increased background sound+ gap+ subject's own tinnitus perception+ startle sound.**

### 9.2.6 Hypothesis for Aim 1 of RQ 3

H0: The self-report rating of how precise the tinnitus is matched with the ongoing background sound is NOT associated with the percentage of startle inhibition in the GPIAS testing paradigm.

H1: The self-report rating of how precise the tinnitus is matched with the ongoing background sound is associated with the percentage of startle inhibition in the GPIAS testing paradigm. The higher the rating of the match between the individual's perceived tinnitus and the sound the individual identifies as the closest match (varying frequency, intensity, and bandwidth) the less startle inhibition would be expected (i.e., the ongoing background sound that is created from the subject's matching procedure would more closely match the actual tinnitus perception, and therefore the tinnitus would more precisely fill in the silent gap).

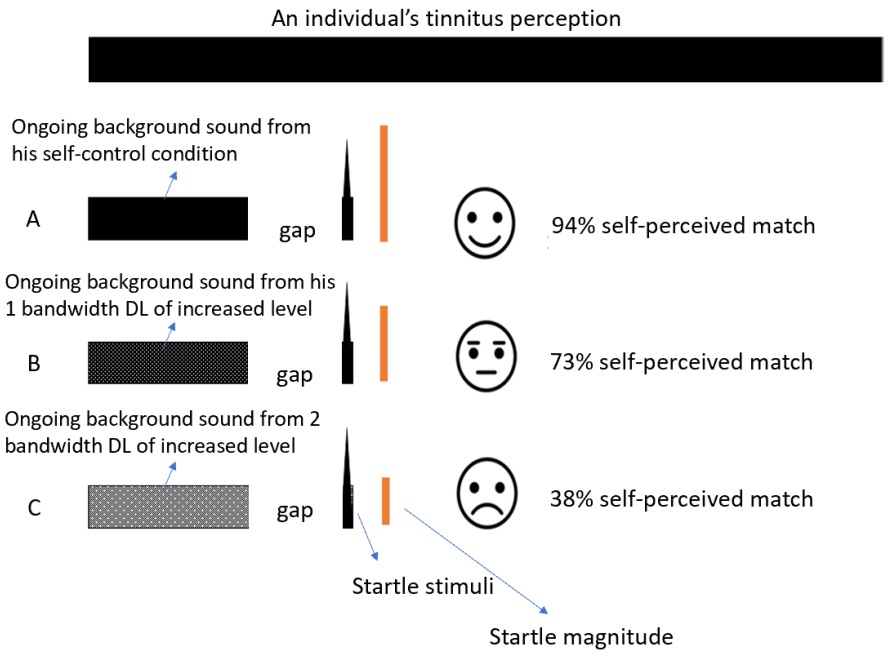



**Figure 28. The self-perceived matching conditions for RQ3. The taller orange bar indicates the larger startle response, while the shorter bars indicate smaller responses (hypothetical results based on H1). The figures on top represented the tinnitus perception of an individual with tinnitus in this study. This participant compared his tinnitus perception with the ongoing background sound from each testing condition. In this illustration, panels A, B and C represent three example conditions. Panels A, B, and C are showing that this individual would rate different scores based on his self-perceived match between tinnitus ongoing background sound from various conditions.**

## 9.3 Experiments

One group of normal-hearing individuals with tonal tinnitus and one group of normal-hearing individuals with no tinnitus were included in the study. Other inclusion/exclusion criteria are listed in **Table 15**. In order to pursue the second and third research questions, the primary

research question had to be answered, which was the purpose of including the individuals with normal hearing and no tinnitus. For the primary research question (*if tinnitus filling in the gap of a GPIAS testing paradigm caused the lack of startle inhibition in individuals with tinnitus as compared to individuals without tinnitus*), it was assumed that for individuals without tinnitus, they would experience a startle inhibition in the condition when there was a silent gap prior to the startle sound (self-control condition, Figure 24, panel D) as compared to the condition when the background sound was played without a silent gap (startle-only condition, Figure 24, panel C). This verifies that startle inhibition was measured when expected in the GPIAS paradigm. For the primary research question, it also was hypothesized that for individuals with tinnitus, they would not experience a startle inhibition in the condition when there was a silent gap prior to the startle sound (self-control condition, Figure 24, panel B) similar to the condition when the ongoing background sound was played without a silent gap (startle-only condition, Figure 24, panel B). This finding supports the hypothesis that the individual's tinnitus would be filling in the gap and therefore would be similar to the startle only condition. If the first two research questions were met, it would mean that the experiment was set up correctly, and the perceived continuous tonal tinnitus was filling in the gap because it was used to create the ongoing background sound (maximally matched to the individual's perceived tinnitus). If both the tinnitus group and the no-tinnitus group had significant startle inhibitions in the self-control condition, or both of the two groups did not show significant startle inhibitions in the self-control condition, then that means it was either that the assumed theory of tinnitus filling in the gap was not legitimate, or the ongoing background sound was not maximally matched with each individual's tinnitus. Because of the uncertainty, we would not proceed to the next step of answering the second and third research questions, since the essence of the rest research questions is established upon the assumed theory

of tinnitus filling in the gap. The expected results for the primary research question were found, thereby the theory of tinnitus filling in the gap was grounded, and the experiment proceeded for the rest research questions.

The second research question (*if deviations in intensity, frequency, or bandwidth of the ongoing background sound as compared to the tinnitus maximally matched background sound could induce startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation in these parameters could be tolerated before an increase in startle inhibition*) and specific aims of the study addressed the allowable range of parameter change that would not produce startle inhibition in people with continuous tonal tinnitus, as well as describing the trend of startle inhibition in people with continuous tonal tinnitus after modifying the signal parameters to a various degree. The parameter changing step was based on the DL of each individual in the tinnitus group. Therefore, participants with tinnitus would serve as their own control. The inhibition percentage from any of the parameter changing levels was compared to the inhibition percentage of the self-control condition for a given individual first to identify a change in startle inhibition.

### 9.3.1 Experimental Procedure

#### 9.3.1.1 Screening Procedure

Participants for both groups read and signed a consent form and completed a Tinnitus history questionnaire to collect demographic information (See Appendix B). Figure 33 displays the experimental configuration. Air conduction thresholds were measured with Grason Stadler (GSI) audiometer in the audiometric sound booth (double-walled, ANSI 2003), following standard

audiometric procedures. Air conducted thresholds were measured using pulsed tones at 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz, 8000 Hz using ER-3A insert earphones, and 10000 Hz, 12500 Hz, and 16000 Hz using Sennheiser HDA 300 headphones. Individuals were included in the study if they had thresholds better than 20 dB HL from 250 to 8000 Hz. Loudness Discomfort Level also was tested to exclude subjects with hyperacusis. Participants with loudness discomfort level at two or more frequencies below 90 dB HL were considered as having hyperacusis (Goldstein & Shulman, 1996) and were excluded from participation.

**Table 14. Classification of Hyperacusis. With permission from Goldstein & Shulman (1996).**

| Hyperacusis | Dynamic Range | Loudness Discomfort Level |
|---|---|---|
| None/Negative | 60 dB or greater all frequencies | 95 dB or greater all frequencies |
| Mild | 50-55 dB at any frequency | 80-90 dB at 2 or more frequencies |
| Moderate | 40-45 dB at any frequency | 65-75 dB at 2 or more frequencies |
| Severe | 35 dB or less at any frequency | 60 dB or lower at 2 or more frequencies |

For both tinnitus group and no-tinnitus group, qualified subjects had normal hearing and had reached the inclusion/exclusion criteria (See Table 15 & Table 16). Participants reporting tinnitus were asked to complete a tinnitus case history questionnaire (See Appendix).

**Table 15. Summary of the inclusion and exclusion criteria of the study**

| Inclusion | Exclusion |
|---|---|
| Inclusion criteria for both groups:<br>o Age between 18 and 55 years<br>o Proficient in English to understand instructions<br>Additional inclusion criterion for tinnitus group:<br>o Participants with bilateral, tonal continuous tinnitus | Exclusion criteria for both groups:<br>o History of medications that may affect inhibition (Table 16)<br>o Any self-reported psychological or brain disorders<br>o Contact lens users that are not willing to take off the lens for this study<br>o Known allergies to skin adhesives and alcohol<br>o Presence of hearing loss and hyperacusis<br>Additional exclusion criterion for tinnitus group:<br>o non-continuous tinnitus<br>o non-tonal tinnitus |

**Table 16. List of the medications that may affect GPIAS (Braff et al., 2001)**

| **Medications found to affect SPIAS, which may potentially affect GPIAS** |
|---|
| ✧ **Ketamine** |
| ✧ **Dopaminergic (Bromocriptine, Haloperidol, Bromocriptine)** |
| ✧ **d-Amphetamine** |
| ✧ **Cocaine withdrawal** |
| ✧ **Nicotine (Cigarette smoking)** |
| ✧ **Ethanol** |
| ✧ **Midazolam** |
| ✧ **Serotonergic (MDMA)** |
| ✧ **Tryptophan** |
| ✧ **Psilocybin** |
| ✧ **Anticholinergics (Procyclidine)** |
| ✧ **Antidepressants (Amitriptyline)** |

#### 9.3.1.2 Tinnitus Identification and Threshold Determination Procedure

For participants with tinnitus (i.e., the tinnitus group), there were two steps for the tinnitus assessment with the Tinnometer (MedRx Co.). Step one was to identify the tinnitus type and maximally match the subject's tinnitus parameters (i.e., frequency, bandwidth, and intensity) with an external sound. In step one, the intensity of tinnitus was labeled as "*Tinnitus Level*" in the Tinnometer. The instruction was given as listed below.

*"Place the earphones on your head, so the center of the earphone is directly over the opening of the ear canal. Make sure the sides (right or left) of the earphones are correct. Tinnitus is experienced by millions of people worldwide. The Tinnometer is designed to mimic the sounds of tinnitus in order to provide an accurate and flexible tinnitus assessment. Please carefully read the following instruction in order to identify your tinnitus.*

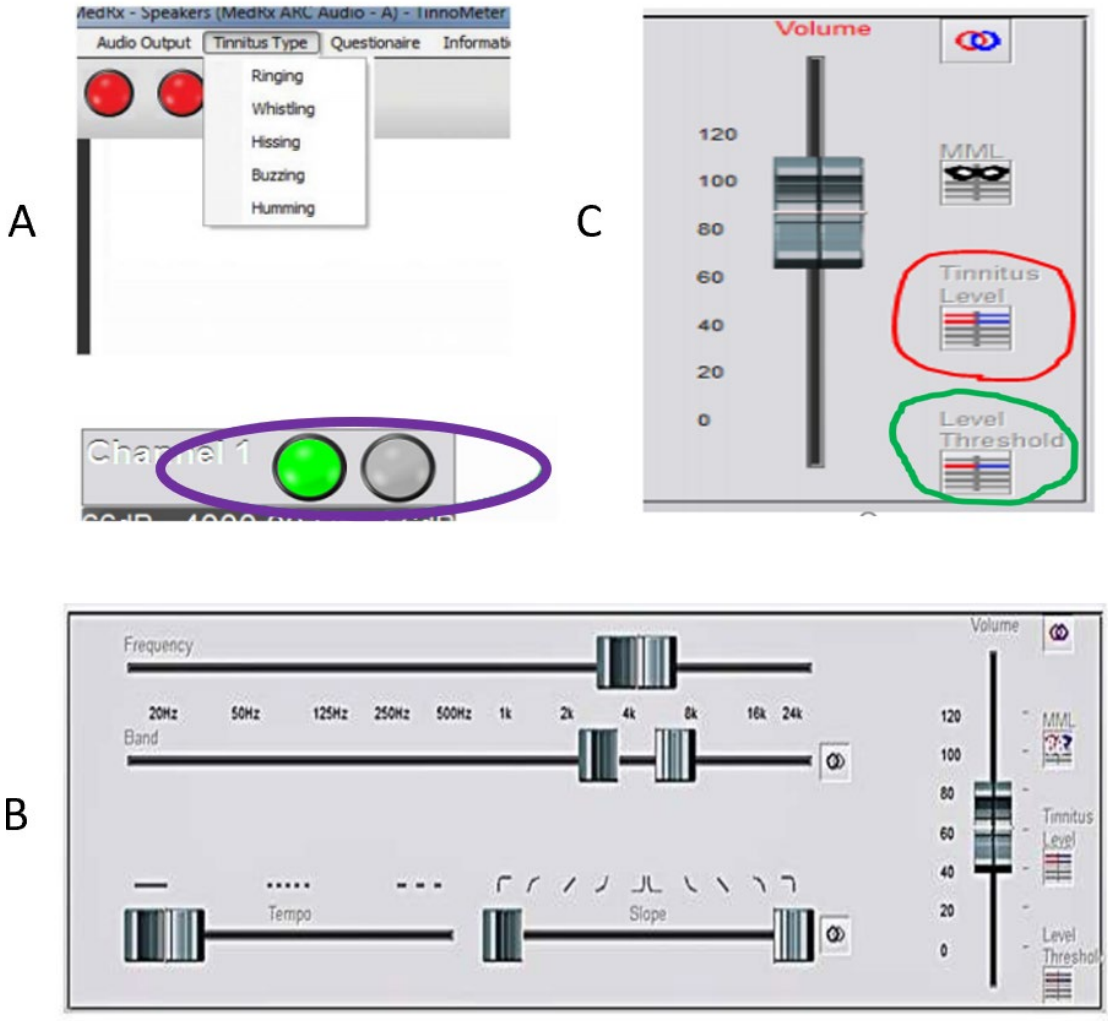



**Figure 29. Tinnitus matching interface in Tinnometer. Panel A is the drop-down manual for choosing tinnitus type, as well as the Start/Stop button in the purple circle. Panel B is the sliders and buttons for tinnitus parameter matching. Panel C is an expansion of a portion of panel B, showing the button for tinnitus loudness (labeled as "Tinnitus Level" in Tinnometer, red circle), and the button for the intensity threshold (labeled as "Level Threshold" in Tinnometer, green circle).**

1. *Start by selecting the tinnitus type that has been described by you (Figure 29, Panel A). This is an approximation of the tinnitus perception you hear. Tinnitus type will provide a starting frequency, bandwidth, and slope to help speed up testing time. <u>If you choose any</u>*

*tinnitus type other than the "Ringing" in the drop-down manual, please stop and let the examiner know.*

2. *Use the stimulus start/stop toggle to present the selected signal to the patient (Figure 29, Panel A, purple circle).*
3. *Use the frequency slider to match the pitch of your perceived tinnitus (Figure 29, Panel B).*
4. *Use the volume slider to match your perceived intensity of tinnitus(Figure 29, Panel B).*
5. *Adjust the bandwidth as needed. For this study, we keep the slope as 100% for every participant. Keep the tempo button on the left as default, but if you have pulsating tinnitus, then please stop and let the experimenter know (Figure 29, Panel B).*
6. *Press the "Tinnitus Level" button (Figure 29, Panel C, red circle) once the intensity of your tinnitus is matched to the external sound played from Tinnometer."*

Step two was to adjust the intensity of the maximally matched external sound to just the audible level, and this threshold was labeled as "*Level Threshold*" in Tinnometer. The reason for measuring the threshold was to calculate the averaged sensation level (SL). The averaged SL of the maximally matched sound from the tinnitus group was used for creating the ongoing background sound of the self-control condition in the no-tinnitus group. The SL of tinnitus maximally matched sound in a participant with tinnitus was calculated following equation #2:

$$\begin{aligned} &The\ sensation\ level\ (SL)\ of\ tinnitus\ maximally\ matched\ sound\ in\ a\ participant\ with\ tinnitus \\ &= This\ participant's\ tinnitus\ intensity - This\ participant's\ threshold \end{aligned} \quad (2)$$

For the no-tinnitus group, subjects also needed to use the Tinnometer but only for the threshold determination of a continuous external sound that shares the averaged frequency and

averaged bandwidth of maximally matched sounds from the tinnitus group. Because in the no-tinnitus group, the ongoing background sound for both startle-only condition and self-control condition was generated based on the averaged central frequency, averaged bandwidth, and averaged SL from the tinnitus group. The intensity of ongoing background sound was calculated based on the following equation #3:

$$\begin{aligned} &\textit{The intensity of ongoing background sound in a participant without tinnitus} \\ &= \textit{This participant's threshold} \\ &+ \textit{Averaged sensation level } (SL) \textit{ of tinnitus maximally matched sound from the tinnitus group} \end{aligned} \tag{3}$$

For participants with tinnitus and without tinnitus, the instruction script on determining threshold was slightly different.

*"Please read the following instruction in order to find the threshold.*

*<u>If you are a participant with tinnitus:</u>*

- *Lower down the intensity of the tinnitus maximally matched sound to the bottom, and then use up arrow key to adjust stimulus intensity until you are just able to hear the sound*
- *Click the "level threshold" button (Figure 29, Panel C, green circle) to get your threshold.*

*<u>If you are a participant without tinnitus:</u>*

- *Lower down the intensity of the sound from Tinnometer to the bottom, and then use up arrow key to adjust stimulus intensity until you are just able to hear the sound*
- *Click the "level threshold" button (Figure 29, Panel C, green circle) to get your threshold."*

For GPIAS experiments, a 100 dB SPL 20Hz ~20KHz broadband (white) noise was adopted as the startle stimuli. Blumenthal & Goode (1991) demonstrated that startle responses could be obtained with broadband stimuli in the range of 50 to 70 dB(A) SPL. However, Berg, Adkinson, & Strock (1973) reported that the 50% probability threshold for a blink response was 85 dB(A) SPL, by measuring lid movement with a mechanical recording device (lid potentiometer) based on a psychophysical threshold determination procedure. Because of this finding, most studies have used startle stimuli higher than 100 dB(A) SPL to reach a higher probability of blink response in humans. Longenecker and Galazyuk (2012) used startle stimuli between 90- and 120-dB SPL, and Fournier and Hebert (2013) used 105 dB(A) SPL startle stimuli, while Hope & Blumenthal (2018) used 100 dB(A) SPL in their studies. The United States Occupational Safety and Health Act standards state that hearing protection is not required for 105 dB(A) SPL stimulus unless the stimulation sound is continuous for 1h. Although people with tinnitus often tend to have hyperacusis, participants with hyperacusis were excluded from participation following the screening procedure in the current study. Based on the above arguments, 100 dB SPL white noise was used as the startle stimuli in the current study.

Sounds used in the experiment were delivered through headphones. Calibration of stimulus intensity was accomplished by the use of a sound pressure level meter placed in the middle of the RadioEar DD450 headphone foam pads based on the dB(A) SPL scale. The startle response was measured with EMG. The Xltek® Protektor32 IOM was used for ocular EMG data collection. The voltage threshold was set to be 0 uV so that even the smallest eye muscle movement could be captured. The time delay for EMG data collection was set at 0 ms in order to avoid any delay in the eye muscle movement tracking.

To accommodate reduced responding across the trials due to short-term habituation, not only were six startle habituation trials played before all testing trials but also the order of the 56 testing trials was fully randomized for each subject (Abel et al., 1998; Braff & Geyer, 2015; Geyer & Swerdlow, 1998). The order of habituation trials and testing trials is illustrated in Figure 30. Each gap embedded trial was composed of 5.88 s of background sound (designed based on the individuals' tinnitus perception as described earlier), 120 ms of the gap, and 50 ms of startle stimuli. Based on each individual's tinnitus parameters, the background sound was generated for the specific individual. The gap was located right before the onset of the startle stimulus. The startle stimulus was a 100 dB (A) SLP white noise signal with an instant rise/fall time (Figure **31**). The above durations of background sound, gap, and startle were based on the study from Peterson & Blumenthal (2018).


**Figure 30. The order of habituation trials and testing trials**

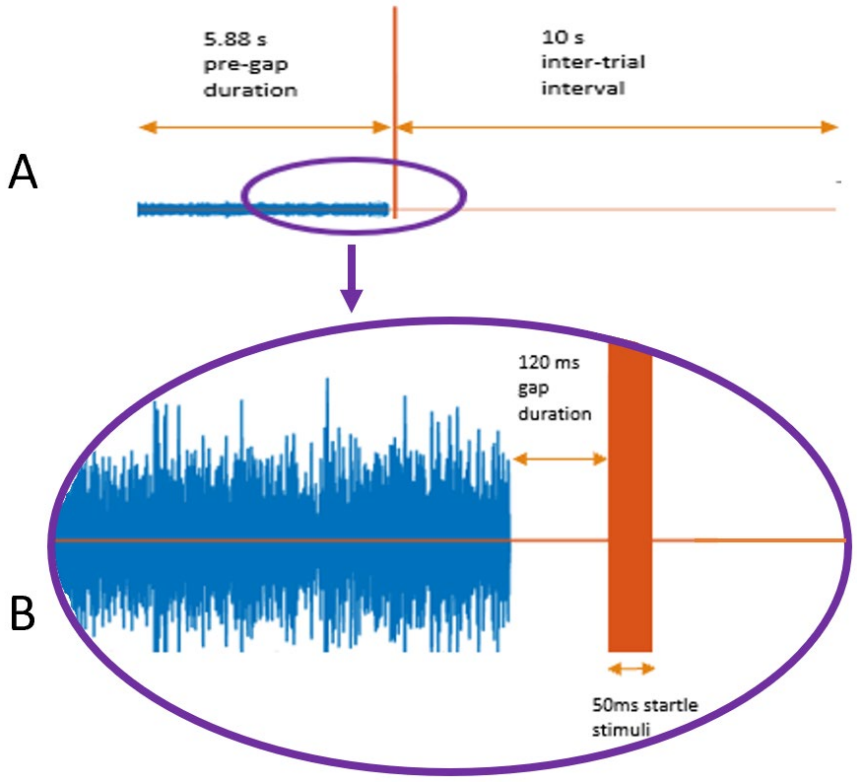



**Figure 31. Illustration of a testing trial. The above is the illustration of a gap condition. Panel B is an expansion of one portion of panel A. Throughout all the gap conditions, there were 5.88 s individualized and ongoing background sound placed right before the 120 ms gap. The startle stimulus was a 50 ms while noise with 100 dB (A) SLP of intensity and an instant rise/fall time. The onset of the startle stimulus was at the end of the gap.**

### 9.3.1.3 DL Determination Procedure

The primary goal was to determine what level of variations in the parameters of frequency, intensity, and bandwidth would not change an individual's startle inhibition magnitude. To answer this question, frequency DL, intensity DL, and bandwidth DL of each individual's tinnitus were measured. The subject listened to the stimuli from the Tinnometer. The stimulus that was the same or maximally close to the tinnitus perception of the subject was identified. The method of

adjustment (Pressey, 1977; Wier, Jesteadt, & Green, 1976) was used for determining DL, in which the subject was in control of the stimulus level and was asked to alter the parameters of stimulus until the subject thought the new stimulus was just noticeably different from their tinnitus perception concerning bandwidth, frequency, and intensity. This process of determining DL was repeated three times for each parameter, and an averaged integer was taken of all of the three values for each parameter. For example, if the values for intensity from three trials were 4, 6, and 5, then the intensity DL for this subject should be (4+6+5)/3 =5 dB SPL.

After the DLs were determined, the testing stimuli that would be used in the GPIAS experiment were generated by the examiner using Matlab and Praat. An example of procedures was as follows. If a subject "A" with tinnitus maximally matched his tinnitus perception with a sound σ (CF of 4000 Hz, 20 dB SL, and a narrow bandwidth +/- 0.19 kHz), then in the startle-only condition and self-control condition, sound σ was given to this subject as ongoing background sound thereby providing the closest match possible to the individual's tinnitus. Afterward, we gave this subject the following 14 conditions with four trials for each condition. The 14 conditions consisted of the startle-only condition (Item 1 in **Figure** 24-26), self-control condition (Item 2 in Figure 24-26), intensity modified conditions with four levels of change (Item 3-6 in **Figure 25**), frequency modified conditions with four levels of change (Item 3-6 in **Figure 26**), as well as bandwidth modified conditions with five levels of DL variations (Item 3-6 in **Figure 27**). All conditions were played in a fully randomized order (Appendix: Table 21). The startle inhibition magnitudes from all of the testing trials were recorded, as reflected by the ocular EMG.

(Example of randomized conditions for subject A with **tinnitus**):

*Condition 1: sound σ + startle (i.e. startle-only condition)*

*Condition 2:* ***2 intensity*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 3:* ***1 frequency*** *DL increased sound σ + gap+ startle stimulus*

*Condition 4:* ***1 intensity*** *DL increased sound σ+ gap+ startle stimulus*

*Condition 5: sound σ (i.e. self-control condition)+ gap+ startle stimulus*

*Condition 6:* ***4 frequency*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 7:* ***1 bandwidth*** *DL increased sound σ+ gap+ startle stimulus*

*Condition 8:* ***3 frequency*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 9:* ***2 intensity*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 10:* ***1.5 bandwidth*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 11:* ***4 intensity*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 12:* ***3 bandwidth*** *DLs increased sound σ+ gap+ startle stimulus*

*Condition 13:* ***2 bandwidth*** *DLs increased sound σ +gap+ startle stimulus*

*Condition 14:* ***3 frequency*** *DLs increased sound σ+ gap+ startle stimulus*

#### 9.3.1.4 GPIAS Experiment Procedure

Ocular electromyogram (EMG) is the most widely used and gold standard measure of the human startle response and was adopted in the current study (Blumenthal, Elden, & Flaten, 2004; Fournier & Hébert, 2013; Lovelace et al., 2006; Shadwick & Sun, 2014). The preparation and experimental procedure of ocular EMG are as follows.

Qualified participants had the skin below their left eye cleaned using a 70% isopropyl alcohol pad. Ag/AgCl electrodes were prepared with high conductivity electrode gel and a miniature adhesive 3M medical tape, which were all adhered to the participant's skin. The skin was cleaned with Nuprep skin prep gel briskly to reduce the impedance. Two recording electrodes

were placed below the lower lid of the eye (Blumenthal et al., 2005), consisting of one electrode placed below the lower eyelid in line with the pupil when looking forward, and another one placed 2cm lateral to the first electrode (Figure 32). A ground electrode was placed on the forehead.

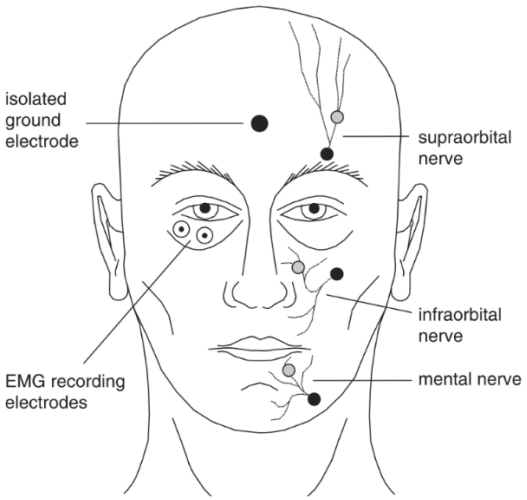



**Figure 32. Electrode montage (with permission from Blumenthal et al. 2005). Left: Placement of EMG recording electrodes over the lower orbital portion of the orbicularis oculi muscle. The isolated ground electrode is placed on the forehead. Right: Electrodes for electrical stimulation of the three main cutaneous branches of the trigeminal nerve.**

Electrode impedances were less than 5KΩ. If the impedance criterion was not met, then electrodes were removed, and the skin preparations and electrode attachment were repeated. Participants were asked to sit still in a chair and look at a target sign placed in front of them during the assessment to control excessive eyeball movement. Each participant was instructed to sit still and listen to the sounds without purposefully being told to blink or not to blink. The subject was encouraged not to pay specific attention to the stimuli during the assessment to alleviate the

attention effect of testing auditory stimuli on the startle inhibition (Filion, Dawson, & Schell, 1993).

*"I will place three electrodes on your face. Two of them will be below your eyeballs, and the third one will be placed on your forehead. During the test, please sit still while avoiding head and body movement. Please just look at the target sign that is in front of you throughout the test and try not to look around the surrounding area. Try to look at the target in your regular way and do not intentionally avoid blinks or do more blinks. At the same time, you will be listening to a series of stimuli that lasts for a few minutes. You do not need to pay specific attention to the stimuli, just relax but remember to only look at the target in front of you."*

The acoustic stimuli were generated from Matlab and Praat and played through the Cakewalk UA-101 Professional 10x10 USB Audio Interface and RadioEar DD450 headphones.

#### 9.3.1.5 Self-perceived Match Rating Procedure

After completing the GPIAS testing, participants with tinnitus were asked to listen to 13 continuous sounds. The series of sounds were randomized following the same randomization order for the GPIAS testing. Each sound was played for 10 seconds. Participants with tinnitus were required to give a matching score to each sound at the end of each playing.

*"In this test, you will be asked to rate and orally report your current feeling of the matching level between your tinnitus perception and the external sound you hear from the computer. The score can vary from anywhere from 0% to 100%, with 0% indicates the least similarity between the two sounds, while 100% indicates the maximum similarity between the two sounds you hear."*

The 13 sounds were the ongoing background sound from each of the all 14 GPIAS testing conditions, including tinnitus maximally matched self-control condition, the conditions at 1/2/3/4

intensity DLs increased level, the conditions at 1/2/3/4 frequency DL(s) increased level, and conditions at the 1/1.5/2/3 bandwidth DL(s) increased level.

### 9.3.1.6 The Summary of the Experimental Procedure

| TINNITUS GROUP | NO-TINNITUS GROUP |
|---|---|
| <u>Screening tasks for participants with tinnitus:</u><br><br>1. Background questionnaire: tinnitus history would be assessed.<br>2. Standard hearing assessment: middle ear function, loudness discomfort level, and the auditory threshold were obtained.<br>3. Tinnitus matching and the difference limens obtained: each participant was asked to use equipment called a Tinnometer to determine the frequency, intensity, and bandwidth of their tinnitus and the just noticeable difference between two stimuli.<br>4. Each participant was asked to use the Tinnometer to determine the threshold of their tinnitus matched stimulus.<br><br>The screening measures took about 40 minutes to complete, after which the participant was informed by | <u>Screening tasks for participants without tinnitus:</u><br><br>1. Standard hearing assessment: middle ear function, loudness discomfort level, and the auditory threshold were obtained.<br>2. The threshold measurement of generated stimuli: each participant would be asked to use equipment called as Tinnometer to determine the threshold of a generated stimulus.<br><br>The screening measures took 20 minutes to complete, after which the participant was informed by the investigator regarding his/her eligibility to participate in this study. |

| | |
|---|---|
| the investigator regarding his/her eligibility to participate in this study. | |
| Experimental Tasks for participants with tinnitus:<br><br>*(For GPIAS testing:)* After screening, the participant had a break while the GPIAS testing stimuli for the experiment were created based on the individual's perception of tinnitus. When the participant came back, he/she was seated comfortably and had electrodes placed above and below the eyes.<br><br>During the test, the participant was asked to sit still and look at a big target sign that was in front of him/her while listening to a series of sounds.<br><br>If the participant had a lot of facial muscle and eyeball movements during the eye muscle tracking measure, the testing was stopped. The participant was given a 5-minute break, and further instructions on controlling his/her movements were provided. Afterward, the same eye muscle tracking measure was restarted for the second time. The participant did not need to pay specific attention to the sounds. The participant looked at the target in front of him/her the whole time.<br><br>*(For self-perceived matching rating:)* At the end of this task, the participant listened to a series of sounds and gave each one a rating of 0~100 in terms of how well it matched the tinnitus. | Experimental Tasks for participants without tinnitus:<br><br>*(For GPIAS testing:)* After the screening, the participant had a break while the GPIAS testing stimuli for this experiment were created based on the averaged central frequency, bandwidth, and sensation level of tinnitus from the tinnitus group, as well as the threshold from this participant. When this participant came back, he/she was seated comfortably and had electrodes placed above and below the eyes.<br><br>During the test, the participant was asked to sit still and look at a big target sign that was in front of him/her while listening to a series of sounds<br><br>If the participant had a lot of facial muscle and eyeball movements during the eye muscle tracking measure, then the testing was stopped. The participant was given a 5-minute break, and further instructions on controlling his/her movements were provided. Afterward, the same eye muscle tracking measure was restarted for the second time. The participant did not need to pay specific attention to the sounds. The participant looked at the target in front of him/her the whole time. |

### 9.3.2 Experimental Configuration

The entire experiment was conducted in a quiet lab room in the Department of Communication Science and Disorders in Forbes Tower at the University of Pittsburgh. See **Figure 33** for the layout of the activities. Outside of the booth, the participants were asked to sit in front of the table 'a.' The laptop A and Tinnometer were located on the table 'a,' where participants characterized their tinnitus perception, determined their individualized difference limen for each tinnitus parameter and rated the tinnitus matching level (i.e., how close the match was between their tinnitus and the sound they had identified as a "match"). Afterward, the participants also were asked to sit in front of the table 'c' where the EMG equipment was located (Figure 33). Table 'c' was where participants participated in the GPIAS experiment. Details of experimental procedures conducted outside and inside of the booth were described in the section of [Experimental procedure].

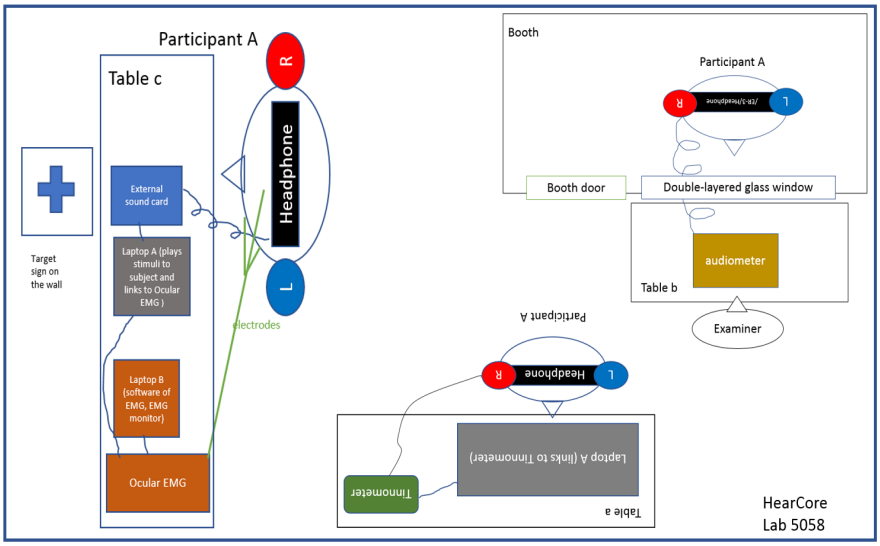



**Figure 33. Experimental configuration**

## 9.4 Ocular Data Analysis

### 9.4.1 Statistical Analysis Outline

IBM SPSS 25 and SAS 9.4 were used for the statistical analysis. For RQ1, the dependent variable was the startle magnitude (as reflected as the ocular EMG blinking amplitude in microvolts), and the independent variable was the condition (self-control condition vs. startle-only condition). The repeated measures ANOVA was used for comparing startle magnitude between the above two conditions within the tinnitus group. Due to the small sample size, Wilcoxon Signed Rank Test was used for comparing startle magnitude between the above two conditions within the no-tinnitus group.

For RQ2, the logarithmized startle inhibition percentage was the dependent variable in this study. The parameter type was the 1$^{st}$ independent variable (IV), and the parameter changing level was the 2$^{nd}$ IV. Parameter type (frequency vs. intensity vs. bandwidth) and the parameter changing level (5 levels) were the independent variables of the study and were analyzed for the main effects. The parameter type × parameter changing level was analyzed for interaction effects. The raw inhibition percentage of a gap condition ＝ (startle magnitude of this specific gap condition － startle magnitude of startle only condition) ／ startle magnitude of startle only condition. Of note, the raw inhibition percentage was logarithmized for normalization (Gerum et al., 2019; Schilling et al., 2017), and therefore the logarithmized startle inhibition percentage was used instead as the dependent variable. The repeated measures ANOVA was applied to answer RQ2 since the transformed data were normally distributed after data transformation.

For RQ3, correlations between the subjective rating of tinnitus matching accuracy, given difference limens of change, and percent Inhibition was analyzed using Spearman rank-order correlation.

### 9.4.2 Sample Size and Power Analysis

In the study from Hope et al. (2018), which was similar in its design to the current study, a 2×3 repeated measures ANOVA was conducted, and the sample size was 57, with eight repeated measures for each condition. There was a nonsphericity correction of $\epsilon = 0.846$, and the alpha level was 0.05. Since the power was not specified in Hope's study, we assumed power of 0.90, and the sensitivity analysis result showed a median effect size f= 0.21. For the current study, a 3×5 repeated measures ANOVA was used, with both the parameter type (frequency vs. intensity vs. bandwidth) and the parameter changing levels (5 levels) as independent variables. The dependent variable was the inhibition level of startle magnitude. Since we would have 14 conditions for each subject with tinnitus, a power analysis using the Gpower computer program (Erdfelder, Faul, & Buchner, 1996) gave a total sample of 25 people with tinnitus needed for detection of a medium effect size with 90% power, a nonsphericity correction $\epsilon = 0.846$ and a 0.5 correlation between repeated measures, at the $\alpha$ level of 0.05. If there were about 15~20% non-responders in people with tinnitus, the sample size we needed was 30 participants in total (Blumenthal et al., 2005). However, although Hope's study was similar to the current study, they were not the same. Therefore, 35 subjects were planned as the sample size for tinnitus group to ensure power. At the same time, a sample size of 10 for the no-tinnitus group would suffice the sample size ratio of 3:1 between subjects with tinnitus and without tinnitus.

## 9.5 Results

### 9.5.1 Data Cleaning

Prior to statistical analysis, any ocular EMG activity occurring with errors related to the experimental procedures and equipment (e.g., electrode failure, computer operational errors) were removed. Non-responders were determined and excluded when the mean startle response of startle-only trials of a participant was <10 units (12.2 μV) (Swerdlow et al., 2007).

Trials with excessive neck and facial muscle movement or significant drowsiness were identified with a criterion of +/- 3 SD from the mean of the startle magnitude of the four startle-only trials in each subject and were removed from the analysis (Chokroverty, Walczak, & Hening, 1992; Hömke, Holler, & Levinson, 2018; Oguro, Aiba, & Hojo, 2001). The startle-only trials should have the largest startle magnitudes in each participant, and therefore, if a participant had a startle magnitude in any gap-embedded trial that was 3 SD above the averaged startle magnitude of startle-only trials, it indicates that a specific gap trial had an abnormally large startle magnitude that could not be explained by normal blink. Such large startle magnitude was often a result of excessive head and neck muscle movements. Similarly, if a gap-embedded trial presented a startle magnitude that was 3 SD lower than the averaged startle magnitude of startle-only trials, then it indicates that the gap trial had an abnormally low startle magnitude that could not be explained by a normal blink as well. Instead, it was often a hint of being overly drowsy. Both situations were not normal, and therefore, those abnormal gap trials were removed.

For the tinnitus group, thirty-seven subjects with tinnitus passed the telephone screening and came in for the study visit. Four people with tinnitus were first excluded from 37 due to hearing loss (N=2), hyperacusis along with hearing loss (N=1), and failed EMG data (N=1). Two more

subjects were excluded from the rest of the 33 subjects from the tinnitus group as non-responders of startle induction. As a result, we had 31 qualified subjects with normal hearing as well as continuous tonal tinnitus in both ears who met the exclusion/inclusion criterion. The non-responder rate among people with tinnitus was 6% (i.e., 2/33) from the current study, which was lower than the expected 15-20%. Blumenthal et al. (2005) indicated that 5-10% of healthy young adults are non-responders. In contrast, rates reported for clinical populations, children, and elderly adults were somewhat higher without a specific rate number, so we ended with an estimate of 15-20% for people with tinnitus considering them as similar to the clinical population. However, the rate from the current data turned out to be 6%, which was the same as that for a healthy young adult population. The reason why we had a lower non-responder rate was likely because the recruited participants were mainly tinnitus noncomplainers with normal hearing, and therefore had acoustic characteristics that were closer to typical healthy hearing people without tinnitus rather than people who came to an audiology clinic due to their hearing complaints.

For the control group, ten subjects without tinnitus passed the phone screening and completed their visits. Two individuals were excluded due to hearing loss (N=1) and failed EMG data (N=1), which ended with eight control subjects qualified for data analysis.

#### 9.5.1.1 EMG Data Processing

Attrition rates due to acoustic non-responders are approximately 5–10% for healthy young adult participants, whereas rates reported for clinical populations are higher than 10% (Blumenthal et al., 2005). The peak of a startle blink response is determined by identifying the maximal EMG value within the time window of 15-150 ms after startle stimulus onset for acoustic blinks (Blumenthal et al., 2005; Braff & Geyer, 2015; Peterson & Blumenthal, 2018). EMG sensitivity was set to be 1.22 μV/unit. A lower than ten units (12.2 μV) startle response of startle-only trials

would indicate a participant exhibited minimal or no startle response to startle-only trials and therefore was categorized as a ‘‘non-responder’’ and this participant’s GPIAS data were excluded (Swerdlow et al., 2007).

Low-pass filtering reduces high-frequency components caused by instrumentation noise and electromagnetic interference. In general, a low-pass filter with a steep roll-off (24 dB per octave or higher) is recommended for EMG signals (Clancy, Morin, & Merletti, 2002). Low-pass filtering with a cutoff frequency at 500 Hz was used (van Boxtel, Boelhouwer, & Bos, 1998), with a sampling rate of 1000 Hz, to generate a bandpass-filtered raw EMG data. As shown in Figure 34, which is an illustrative trace of an acoustic startle response evoked by a 100 dB(A) SPL startle stimulus (white noise). Within the time window of 15-150 ms after the onset of the startle stimulus in each trial, 600 peaks and troughs with the largest amplitudes were captured automatically and output in the format of Excel by the built-in software of Xltek® Protektor32 IOM. After pulling out the peaks and troughs, the bandpass-filtered raw EMG data were full-wave rectified, which means the thoughs were turned into peaks, while the original peaks were kept as peaks still. Thereby, the 600 peaks and troughs were turned into 600 peaks only. Afterward, the peak with the maximal EMG amplitude was determined as the startle response for the specific trial (green arrow,Figure 34). The amplitude of startle response was termed as **startle magnitude**.

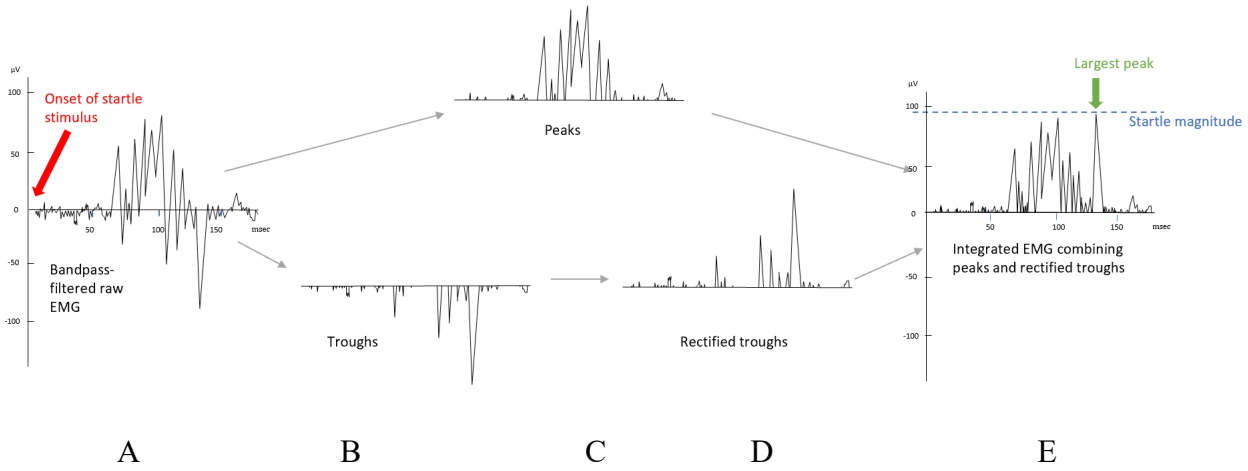



**Figure 34. Illustrative tracing of an acoustic startle response evoked by a 100 dB(A) SPL startle stimulus. Panel A is the bandpass-filtered raw EMG. Panel B shows the troughs only with Panel D showing these data rectified. Panel C shows the peaks only. Panel A is the integrated EMG combining the peaks and rectified troughs. The startle magnitude that is used in the final analysis is the highest peak from panel E in the time window between 15-150 ms.**

The Figure 34 provides an illustration of the EMG tracing of a startle response including the manipulation that is required to obtain the peak startle response for an individual. The first tracing (A) depicts the bandpass-filtered raw EMG. The 100 dB SPL startle signal included in the GPIAS paradigm was presented at time point 0 ms on this graph (red arrow). The top tracing (C) depicts the peaks of an ocular EMG response, and the bottom tracing (B and D) depict the original troughs (B) and rectified troughs (C) of an ocular EMG response. The tracing to the far right (E) depicts the integrated EMG, which combines the peaks and rectified troughs. The Y-axis of all tracings is in microvolts. The X-axis represents the time from the startle stimulus onset in milliseconds. The EMG tracing displays spikes of increasing amplitude that peak at around 130 ms after the onset of a startle stimulus. The dotted blue line indicates the startle magnitude, which

matches the amplitude of the absolute value of the largest peak (green arrow) within the time window of 15-150 ms after the onset of a startle stimulus.

There were four trials for each condition and the absolute value of the largest peak of each trial was averaged across the four trials. The average EMG peak values from the startle-only condition (when no gap was present and therefor no inhibition is expected) and the average EMG peak values from the conditions of interest in the study (e.g., self-control condition, conditions with four parameter -changed levels) were used to calculate raw inhibition percentage for each condition of interest.

For the primary research question (does tinnitus filling in the gap of a GPIAS testing paradigm cause the lack of startle inhibition in individuals with tinnitus as compared to individuals without tinnitus), the startle magnitude (described above) from each condition was used as the dependent variable. When using repeated measures ANOVA to compare startle magnitude between the self-control condition and the startle-only condition within the tinnitus group, the dependent variable was startle magnitude and the two independent variables were condition and trial. For the same comparison within the no-tinnitus group, the Wilcoxon Signed Rank Test was used due to a small sample size, while the dependent variable was the averaged startle magnitude from 4 trials, along with the same independent variable of condition (self-control vs. startle-only).

For the second research question which assessed the impact of deviating the background sound from the individual's perceived tinnitus on parameters of intensity, frequency, and bandwidth, the logarithimized startle inhibition was used in the analysis. A description of how the logarithmized startle inhibition was calculated is included below.

The equation for calculating raw inhibition percentage of any gap condition was as follows:

$$percentage\ of startle\ inhibition\ in\ gap\ condition =$$

$$\frac{startle\ magnitude\ of\ gap\ condition - startle\ magnitude\ of\ startle\ only\ condition}{startle\ magnitude\ of\ startle\ only\ condition}$$

In this equation, the gap conditions for the current study include the self-control condition, 1- 4 intensity DLs increased conditions, 1- 4 frequency DLs increased conditions, 1- 3 bandwidth DLs increased conditions. To calculate an inhibition percentage, the averaged startle magnitude from four trials in each condition are calculated as the first step. For instance, a subject's startle magnitude for 1 intensity DL of increase level (a gap condition) was 99 μV(trial 1), 100 μV (trial 2), 97 μV (trial 3), 96 μV (trial 4) from four trials. Therefore, the averaged startle magnitude for this gap condition would be 98 μV = (99+100+97+96)/4. We then need this subject's averaged startle magnitude for his startle-only condition, which was 110 μV as an example. Then based on the above equation for calculating inhibition percentage, the inhibition percentage at the one intensity DL of increased level would be (98-110)/110= -0.11, which means there was 11% decrease in startle magnitude at this specific level as compared to the startle magnitude in the startle-only condition for this subject. Since the raw inhibition percentage was negative, a constant of 1 was added to the value for logarithimization. In this example, the **logarithmized inhibition percentage** would be Log(-0.11+1) = -0.051. The logarithmized inhibition percentage for each condition was then subjected to a repeated-measures ANOVA (see section 9.4.1); setting the logarithmized inhibition percentage as a dependent variable and level as an independent variable (4 parameter-changed levels + self-control condition as the original level).

### 9.5.2 Demographic Data

*The comparison of demographics between the no-tinnitus group and the tinnitus group*

The hearing thresholds for both no-tinnitus group (N=8) and tinnitus group (N=31) from 250 Hz to 8 kHz and 10 kHz to16 kHz are in Figure 36. Tinnitus parameters for subjects with tinnitus are listed in Table 19. Among the 40 subjects, 30 subjects with tinnitus and nine subjects without tinnitus had complete hearing threshold tests, except for one subject who had no extended high-frequency threshold because of an equipment issue. Due to the unequal sample size between groups, nonparametric measures were used to test statistical differences. A Mann–Whitney U test was performed to determine any significant between-group difference for demographics. Significant difference was found for the clinical PTA thresholds (.5, 1, 2 kHz) between the control and tinnitus groups in the right ($U = 61$, $z = -2.563$, $p = 0.009$), but not in the left ear ($U = 85$, $z = -1.777$, $p = 0.077$), indicating a better hearing threshold in the right ear of control subjects. There was neither any difference between groups regarding EHF PTA thresholds (10 12.5 16 kHz) in the right ear (U=131.5, Z=-0.117, P=0.915) nor in the left ear (U=125, Z=-0.334, P=0.749). To clarify, due to the maximum output limit of our high-frequency audiometer (35 dB HL) at 16 kHz, several subjects had no response at 16 kHz (**Table 16**). Such "no response" at EHF range and the related data loss for hearing threshold analysis is common, although it may not be specified in published papers (Campbell, Bean, & LaBrec, 2018; Shim et al., 2009; Somma et al., 2008). These data could either be excluded (Campbell et al., 2018) or be extrapolated for analysis (Somma et al., 2008). For the current study, the threshold of these non-responders at 16 kHz was extrapolated (following a 5 dB step) as 40 dB HL as the hearing threshold for the subjects for statistical analysis.

Although there were no differences in EHF thresholds in our current study, which matches results from Campbell et al. (2018), there have been inconsistent findings regarding the EHF

thresholds in people with tinnitus. Some studies have reported no difference between people with and without tinnitus concerning their EHF thresholds. At the same time, some other earlier findings showed an elevated EHF threshold in those with tinnitus compared to the people without tinnitus. Campbell et al.'s study (2018) was conducted in subjects with normal hearing as well as tinnitus, and subjects with normal hearing but without tinnitus. The current study has participants with similar characteristics to those in Campbell et al.'s study.

Reduced auditory input to these high-frequency regions might initiate a decrease in central inhibition, which might then contribute to the tinnitus percept in this population (Eggermont & Roberts, 2015; Noreña & Farley, 2013). We wanted to explore how the EHF thresholds correlated with tinnitus from our observation. Although Campbell et al. 's study implicitly excluded those who had no responses at EHF for analysis, while the current study included those participants, both studies did not see a significant difference in EHF thresholds between people with and without tinnitus when all of them had clinically normal hearing (Campbell et al., 2018).

**Table 17. Number of subjects based on the group, ear, and threshold value at 16 kHz.**

| | <=5 DB HL | 6~15 DB HL | 16~25 DB HL | 26~35 DB HL | >35 DB HL |
|---|---|---|---|---|---|
| **R TINNITUS** | 6 | 4 | 3 | 7 | 11 |
| **L TINNITUS** | 3 | 7 | 6 | 6 | 8 |
| **R CONTROL** | 1 | 1 | 0 | 2 | 5 |
| **L CONTROL** | 1 | 0 | 1 | 1 | 6 |

The LDL thresholds were significantly reduced in both ears and at all frequencies in the tinnitus group as compared to the no-tinnitus group (Table 18). This result indicates that participants without tinnitus tended to have better tolerance of loudness compared to participants

with tinnitus (Figure 35 ). No age difference between the tinnitus group (M= 31.8, SD= 13.4) and the no-tinnitus group (M=32.2, SD=10.1), t (17.090)=0.093, P=0.927 (Figure 37). Gender distribution was evenly laid out between females and males in both groups (Figure 37).

**Table 18. Test statistics of LDL between tinnitus and no-tinnitus groups by ear side across frequencies**

| TEST STATISTICS | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | R 250 | R 500 | R 1000 | R 2000 | R 4000 | L 250 | L 500 | L 1000 | L 2000 | L 4000 |
| Mann-Whitney U | 63.500 | 76.500 | 58.500 | 78.000 | 87.000 | 26.000 | 49.000 | 30.500 | 44.000 | 76.000 |
| Z | -2.963 | -2.098 | -2.682 | -2.049 | -1.733 | -4.161 | -3.017 | -3.593 | -3.152 | -2.090 |
| P | .002 | .035 | .006 | .041 | .085 | .000 | .002 | .000 | .001 | .035 |

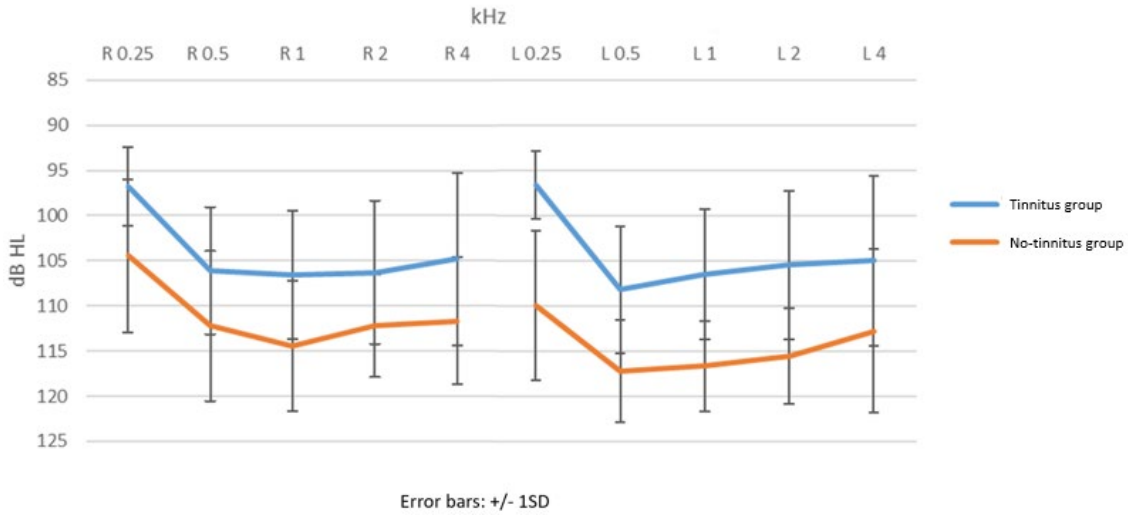



**Figure 35. Averaged LDL of participants with tinnitus.**

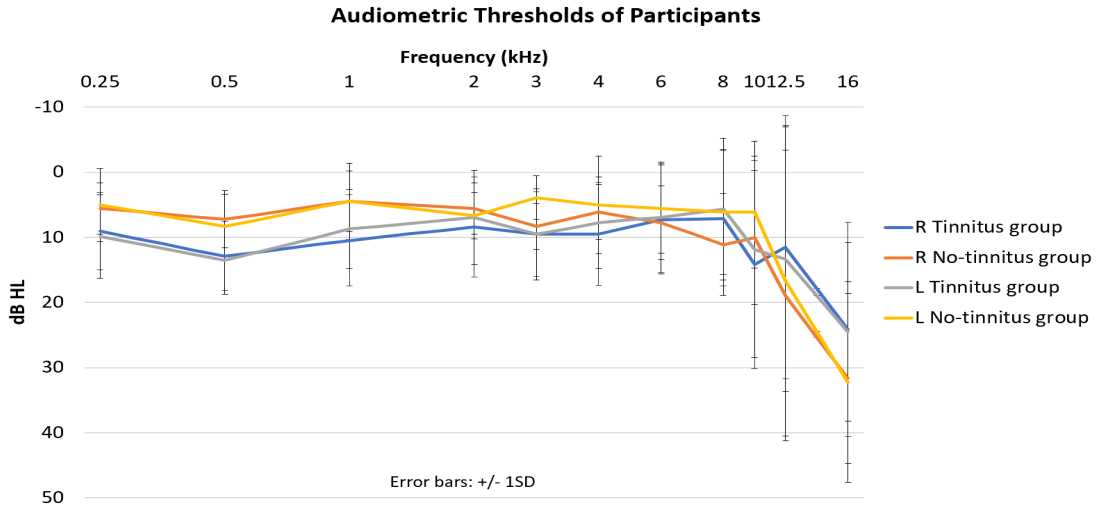



**Figure 36. Participants' average audiometric thresholds (with ±1 SD ). R Tinnitus: frequencies 0.25 – 8 kHz (N=31), 10 - 12 kHz (N=30), 16 kHz (N=20). R Control: frequencies 0.25 – 8 kHz (N=8), 10 - 12 kHz (N=8), 16 kHz (N=4). L Tinnitus: frequencies 0.25 – 8 kHz (N=31), 10 - 12 kHz (N=30), 16 kHz (N=23). L Control: f frequencies 0.25 – 8 kHz (N=8), 10 - 12 kHz (N=8), 16 kHz (N=3).**

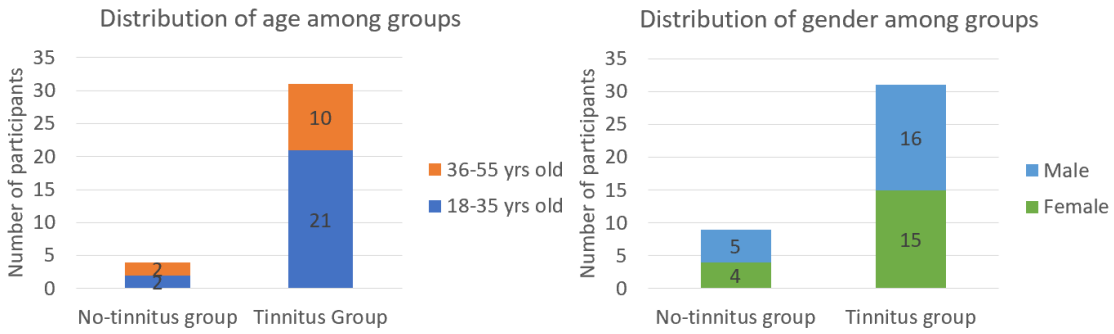



**Figure 37. Distribution of age and gender between groups.**

**Table 19. Tinnitus Parameters of 31 Subjects with Tinnitus.**

| ID # | original CF | original bandwidth in octave (lower boundary) | original bandwidth in octave (higher boundary) | the initial intensity in SPL | altered central frequency | altered bandwidth in octave (lower boundary) | altered bandwidth in octave (higher boundary) | the altered intensity in SPL | bandwidth DL in octave | intensity DL in SPL | frequency DL in Hz |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 1449 | 0.33 | 0.33 | 52 | 1486 | 0.57 | 0.57 | 54 | 0.24 | 2 | 37 |
| 4 | 2561 | 0.3 | 0.3 | 64 | 2782 | 0.63 | 0.63 | 72 | 0.66 | 8 | 221 |
| 5 | 5774 | 0.01 | 0.01 | 43 | 5874 | 0.12 | 0.12 | 46 | 0.22 | 3 | 100 |
| 6 | 4198 | 0.17 | 0.17 | 53 | 4353 | 0.28 | 0.28 | 59 | 0.22 | 6 | 155 |
| 7 | 6683 | 0.2 | 0.2 | 43 | 7838 | 0.41 | 0.41 | 47 | 0.42 | 4 | 1155 |
| 8 | 6772 | 0.01 | 0.01 | 53 | 6955 | 0.3 | 0.3 | 61 | 0.58 | 8 | 183 |
| 9 | 2390 | 0.02 | 0.02 | 42 | 2469 | 0.31 | 0.31 | 48 | 0.58 | 6 | 79 |
| 10 | 6595 | 0.45 | 0.45 | 47 | 7419 | 0.81 | 0.81 | 51 | 0.72 | 4 | 824 |
| 12 | 5140 | 0.24 | 0.24 | 50 | 5225 | 0.34 | 0.34 | 54 | 0.2 | 4 | 85 |
| 14 | 7048 | 0.01 | 0.01 | 52 | 8377 | 0.43 | 0.43 | 59 | 0.84 | 7 | 1329 |
| 15 | 8377 | 0.51 | 0.51 | 44 | 8474 | 0.55 | 0.55 | 46 | 0.08 | 2 | 97 |
| 16 | 9316 | 0.06 | 0.06 | 66 | 9562 | 0.08 | 0.08 | 67 | 0.04 | 1 | 246 |
| 17 | 4001 | 0.08 | 0.08 | 40 | 4312 | 0.42 | 0.42 | 46 | 0.68 | 6 | 311 |
| 18 | 6491 | 0.13 | 0.13 | 44 | 6754 | 0.21 | 0.21 | 46 | 0.16 | 2 | 263 |
| 20 | 9275 | 0.01 | 0.01 | 46 | 10035 | 0.22 | 0.22 | 49 | 0.42 | 3 | 760 |
| 21 | 5384 | 0.22 | 0.22 | 51 | 5584 | 0.48 | 0.48 | 54 | 0.52 | 3 | 200 |
| 22 | 8266 | 0.27 | 0.27 | 53 | 8290 | 0.5 | 0.5 | 58 | 0.46 | 5 | 24 |
| 23 | 4959 | 0.02 | 0.02 | 32 | 5113 | 0.42 | 0.42 | 38 | 0.8 | 6 | 154 |
| 24 | 4253 | 0.01 | 0.01 | 32 | 4370 | 0.23 | 0.23 | 36 | 0.44 | 4 | 117 |
| 25 | 8489 | 0.02 | 0.02 | 40 | 8495 | 0.11 | 0.11 | 41 | 0.18 | 1 | 6 |
| 26 | 6595 | 0.08 | 0.08 | 67 | 7142 | 0.12 | 0.12 | 71 | 0.08 | 4 | 547 |
| 27 | 6863 | 0.05 | 0.05 | 50 | 7075 | 0.35 | 0.35 | 55 | 0.6 | 5 | 212 |
| 28 | 8215 | 0.03 | 0.03 | 48 | 8275 | 0.06 | 0.06 | 52 | 0.06 | 4 | 60 |
| 29 | 7080 | 0.08 | 0.08 | 68 | 7090 | 0.1 | 0.1 | 72 | 0.04 | 4 | 10 |
| 30 | 2277 | 0.5 | 0.5 | 45 | 2327 | 0.63 | 0.63 | 47 | 0.26 | 2 | 50 |
| 31 | 4188 | 0.26 | 0.26 | 43 | 4194 | 0.4 | 0.4 | 45 | 0.28 | 2 | 6 |
| 32 | 3008 | 0.21 | 0.21 | 64 | 3060 | 0.26 | 0.26 | 65 | 0.1 | 1 | 52 |
| 33 | 4253 | 0.01 | 0.01 | 41 | 4257 | 0.04 | 0.04 | 45 | 0.06 | 4 | 4 |
| 35 | 5055 | 0.04 | 0.04 | 64 | 5120 | 0.08 | 0.08 | 67 | 0.08 | 3 | 65 |
| 36 | 8329 | 0.02 | 0.02 | 64 | 9285 | 0.04 | 0.04 | 67 | 0.04 | 3 | 956 |
| 37 | 6595 | 0.08 | 0.08 | 63 | 6601 | 0.14 | 0.14 | 65 | 0.12 | 2 | 6 |

*The demographics within the tinnitus group*

As shown in Figure 37, the final analysis of the tinnitus group included 31 qualified participants, including 15 males and 16 females. The mean age was 31.83 Twenty-one of them reported no family history of tinnitus, while the rest 10 reported positive family history that their parents had had tinnitus. The average initial onset was 18.4 years old, except for age from two subjects who failed to report certain year/age for tinnitus onset. Twenty-five participants reported a gradual onset, while six people reported an abrupt onset.

All qualified subjects experienced bilateral tinnitus, with twelve of them indicating that the tinnitus in both ears was equal. Six of the subjects reported the tinnitus being worse in the left ear, while nine reported tinnitus being worse in the right ear, along with four reporting tinnitus inside of the head. The average scale of the loudness of tinnitus was 43.2 out of 100. Moreover, people were aware of their tinnitus for 50.1 % of their time on average. When asked whether their tinnitus could be reduced by music or by certain types of environmental sounds, twenty-two said yes, and six said no, while three said they did not know. Concerning the question of "Does any head and neck movement (e.g., moving the jaw forward or clenching the teeth), or having your arms/hands or head touched, affect your tinnitus?", twenty-three said no, while eight people said yes. Although none of these participants had reduced LDLs based on clinical norms, only eight participants said they never had a problem tolerating sounds. Fifteen of them rarely reported having a problem with loud sounds. At the same time, six said they sometimes had trouble tolerating loudness, and two of them reported an intolerance of loud sound frequently.

To further understand any potential LDL differences among participants with four different self-reported loudness intolerance levels, participants were put into four groups (Figure 38), and a

Kruskal Wallis analysis was conducted. There was no significant difference in LDLs across frequencies between these four groups of participants (Figure 38).

**Table 20. Test statistics of LDL between participants with different self-report of loudness intolerance level. The question of loudness intolerance level was asking, "Do you have a problem tolerating sounds because they often seem much too loud?" Subjects have to give an answer choosing from "never," "rarely," "sometime," or "usually." The self-reported answers were used as a grouping variable to compare their LDL across frequencies by ear side.**

| TEST STATISTICS * | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | R 250 | R 500 | R 1000 | R 2000 | R 4000 | L 250 | L 500 | L 1000 | L 2000 | L 4000 |
| Kruskal-Wallis H | 4.045 | 4.249 | 4.650 | 6.741 | 2.736 | 3.929 | 3.728 | 2.533 | 2.199 | 2.873 |
| df | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| P | .272 | .242 | .201 | .066 | .463 | .266 | .307 | .496 | .562 | .443 |

*. Grouping Variable: self-report of loudness intolerance level

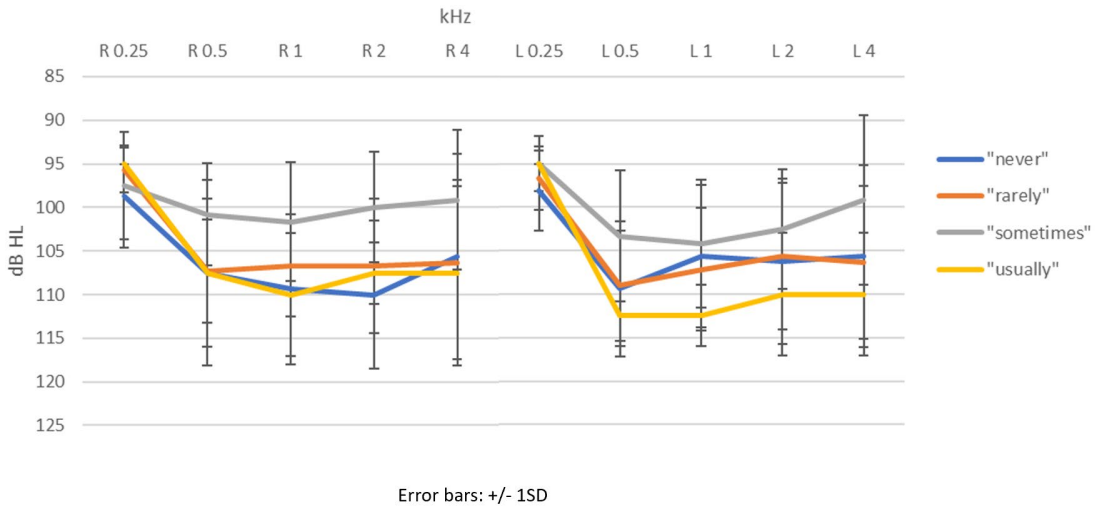



**Figure 38. LDL grouped by four self-reported loudness intolerance levels in participants with tinnitus. The self-reported answers were used as a grouping variable to compare their LDLs across frequencies for right and left ears.**

### 9.5.3 The Primary Research Questions: If Tinnitus Filling in the Gap Was the Cause for the Lack of Inhibition in Individuals with Continuous Tonal Tinnitus as Compared to Individuals without Tinnitus

The primary research question was whether tinnitus filling in the gap was the cause for the lack of inhibition in individuals with continuous tonal tinnitus as compared to individuals without tinnitus. The primary research question needed to be answered first to make sure the experiment set up was correct and could be used to further answer the second research question (*if deviations in intensity, frequency, or bandwidth of the ongoing background sound as compared to the tinnitus maximally matched background sound could induce startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation in these parameters could be tolerated before an increase in startle inhibition*). For specific aim 1 of the primary research question, it was assumed that in

individuals with no tinnitus, a startle inhibition would be measured when there was a silent gap presented (self-control condition) in the GPIAS paradigm as compared to when the ongoing background sound was played without a gap presented (startle-only condition). For specific aim 2 of the primary research question, it was assumed that in individuals with tinnitus, there would be no startle inhibition in the self-control condition (silent gap presented with the assumption that tinnitus was filling in the gap). Only based on the above assumed results could the rest research questions be further explored.

To test if there would be startle inhibition in the self-control condition in both the tinnitus group and no-tinnitus group (i.e., specific aim 1 and aim 2 of primary research questions), the magnitudes of startle response between startle-only condition and self-control condition were compared within each group. Due to the small sample size, the distribution of EMG data from the no-tinnitus group was non-normal even after logarithmization. Therefore, the Wilcoxon Signed Rank Test was used for the post hoc analysis within the no-tinnitus group. Only 1/8 subjects without tinnitus (12.5%) failed to show startle inhibition at the self-control condition, and post hoc analysis in the no-tinnitus group showed a significantly decreased magnitude of the startle response in self-control condition as compared to the startle only condition, $Z=-2.558$ $P=0.010$. In contrast to the no-tinnitus group, 10/31 subjects with tinnitus (32.3%) showed lack of startle inhibition at the self-control condition, and post hoc analysis using repeated measures ANOVA within the tinnitus group showed no significant difference in the magnitude of startle response between self-control condition and startle only condition (Figure 39), $SE=0.944$, $P=0.141$.

Since the assumed results for the primary research questions were found, there should be a lack of startle inhibition in the self-control condition in the tinnitus group as compared to the same condition in the no-tinnitus group. This was validated by comparing the inhibition percentage

in the self-control condition between the no-tinnitus group (N=8) and the tinnitus group (N=31). The Mann-Whitney U test was used for analysis due to the unequal sample sizes between groups. In the analysis, the dependent variable was the inhibition percentage, and the independent variable was the group. This between-group analysis showed significantly less inhibition in the tinnitus group as compared to the no-tinnitus group on the self-control condition (Figure 39), U = 2272.00, Z = -2.602, P = 0.009.

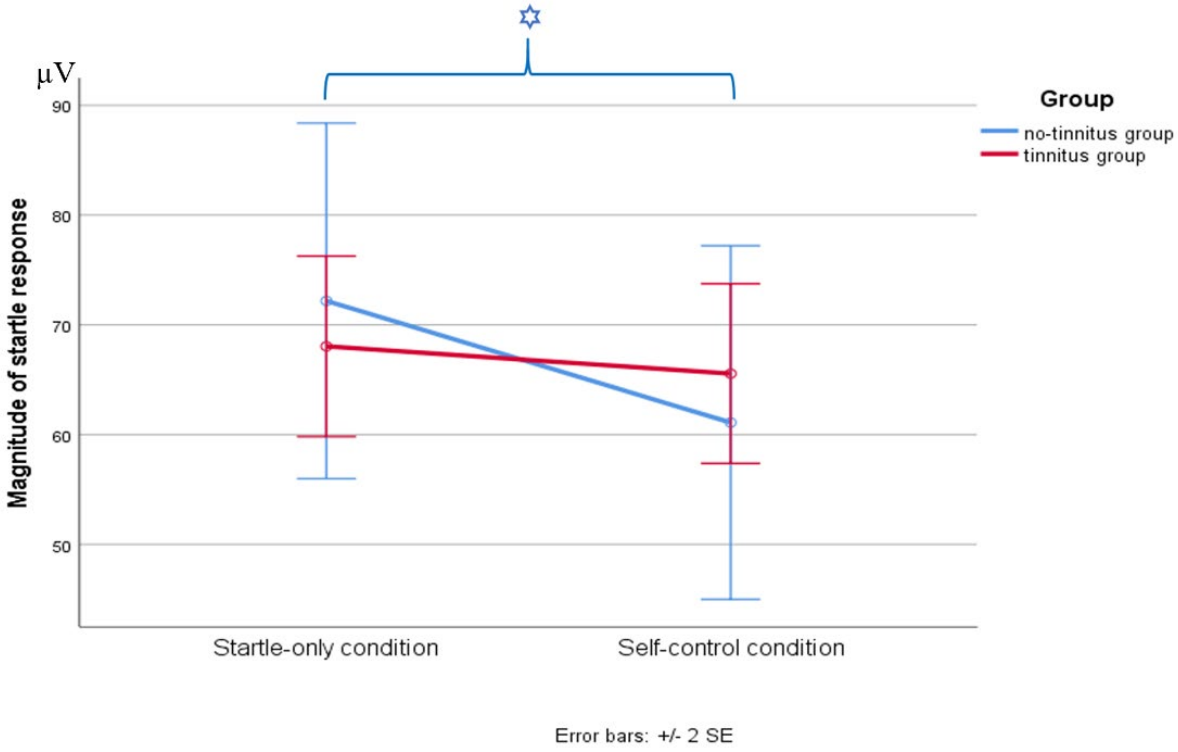



**Figure 39. The magnitude of startle response between participants with and without tinnitus. This blue star represents a significant decrease in startle response at the self-control condition as compared to the startle-only condition that occurred in the no-tinnitus group. No such difference was observed between the two conditions in the tinnitus group.**

### 9.5.4 The Second Research Question: If Deviations in Intensity, Frequency, or Bandwidth of the Ongoing Background Sound as Compared to a Background Sound Maximally Matched to an Individual's Tinnitus Perception Could Induce Startle Inhibition Change In the GPIAS Testing Paradigm. If so, What Range of Deviation Would Not Lead to Startle Inhibition.

The second research question (**RQ2**) examined whether deviations in intensity, frequency, or bandwidth of the ongoing background sound as compared to the tinnitus maximally matched background sound could induce startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation in these parameters could be tolerated before an increase in startle inhibition.

Only individuals with tinnitus (N=31) were included in this analysis. The repeated-measures ANOVA was applied for the analysis of the data. In the analysis, the within-subject factors were the parameter changing levels and parameter types, and the logarithmic inhibition percentage was analyzed as the dependent variable. The Mauchly's test of sphericity showed a significant violation of sphericity, P <0.001. If the Epsilon from the sphericity test was < 0.75, the sphericity correction should be based on the Greenhouse-Geisser correction method. Since the Epsilon for parameter changing levels was 0.719, the Greenhouse-Geisser correction method was used. A significant main effect of parameter type was observed within the tinnitus group, $\eta_p^2$= 0.025, F (1.268, 195.319) = 4.028, P = 0.037, indicating changing different types of parameters affected the inhibition differently (Figure 40). The main effect of the parameter changing level also was found within the tinnitus group, $\eta_p^2$= 0.043, F (2.201, 338.899)=7.000, P=0.001. Along

with the two significant main effects, an interaction effect of parameter type × parameter changing level was found as well, $\eta_p^2$= 0.031, F (4.569, 703.665) = 4.972, P<0.001.

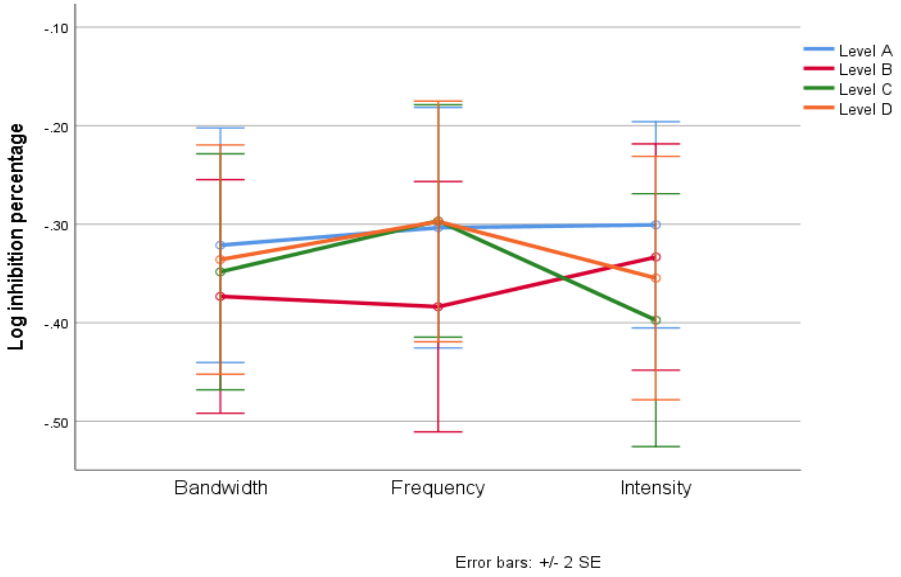



**Figure 40. Interaction effect as the function of logarithmized inhibition percentage within the tinnitus group**

A post hoc analysis was conducted within each parameter type to understand the different inhibition patterns within each parameter.

### 9.5.4.1 Intensity (Aim 1 and Aim 4 of RQ 2)

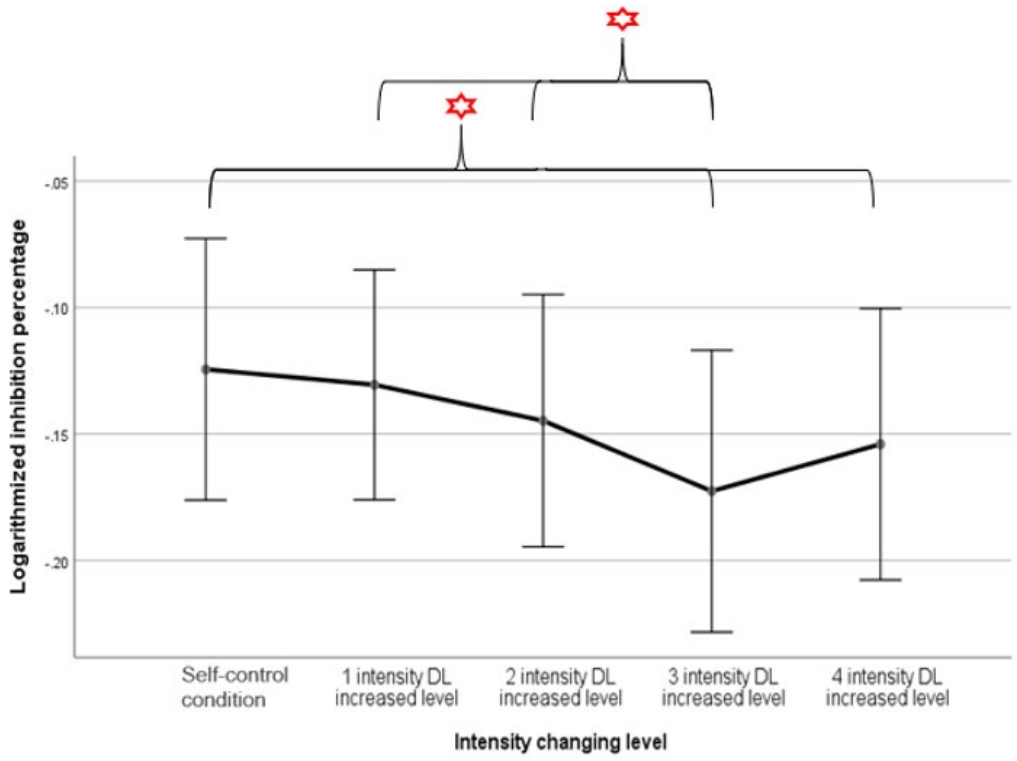
There were five parameter-changing levels in intensity (**Figure 25**), including the tinnitus matched self-control condition, one intensity DL increased level, two intensity DLs increased level, three intensity DLs increased level, and four intensity DLs increased level.

**Aim1** of RQ2 was to explore if variation in intensity (See items 3, 4, 5, 6 in **Figure 25**) of the ongoing background sound leads to startle inhibition change as compared to the tinnitus

matched self-control condition (See item 2 in **Figure 25**). Part of **Aim4** was to understand within what level of variations in the intensity of the ongoing background sound, will there be no significant change in startle inhibition.

To reach Aim1 & Aim4, post hoc analysis was conducted using repeated measures ANOVA on logarithmized inhibition percentage within the data set of intensity in the tinnitus group. Based on Greenhouse-Geisser correction, a significant difference in the percentage of startle inhibition existed across different levels of intensity change, F (3.034, 467.177)= 10.097, $\eta_p^2$= 0.062, P < 0.001.

Pairwise comparisons of logarithmized startle inhibition percentage among levels showed significant difference between the self-control condition and the 3 DL increased level (mean difference =0.048, P<0.001, SE = 0.006), as well as between self-control condition and the 4 DL increased level (mean difference =0.030, P<0.001, SE=0.006), but no statistical difference between 3 DL and 4 DL (mean difference = - 0.019, P= 0.139, SE= 0.007) (Figure 41).




| | |
|---|---|
| Startle was NOT inhibited as compared to self-control condition | **Startle was inhibited as compared to self-control condition** |
| 1 & 2 intensity DL(s) increased level | 3 & 4 intensity DLs increased level |

**Figure 41. Logarithmized inhibition percentage across intensity changing levels. The red star indicates a statistical difference. Inhibition percentage in any gap condition = (startle magnitude of this gap condition − startle magnitude of startle only condition) / (startle magnitude of startle only condition).**

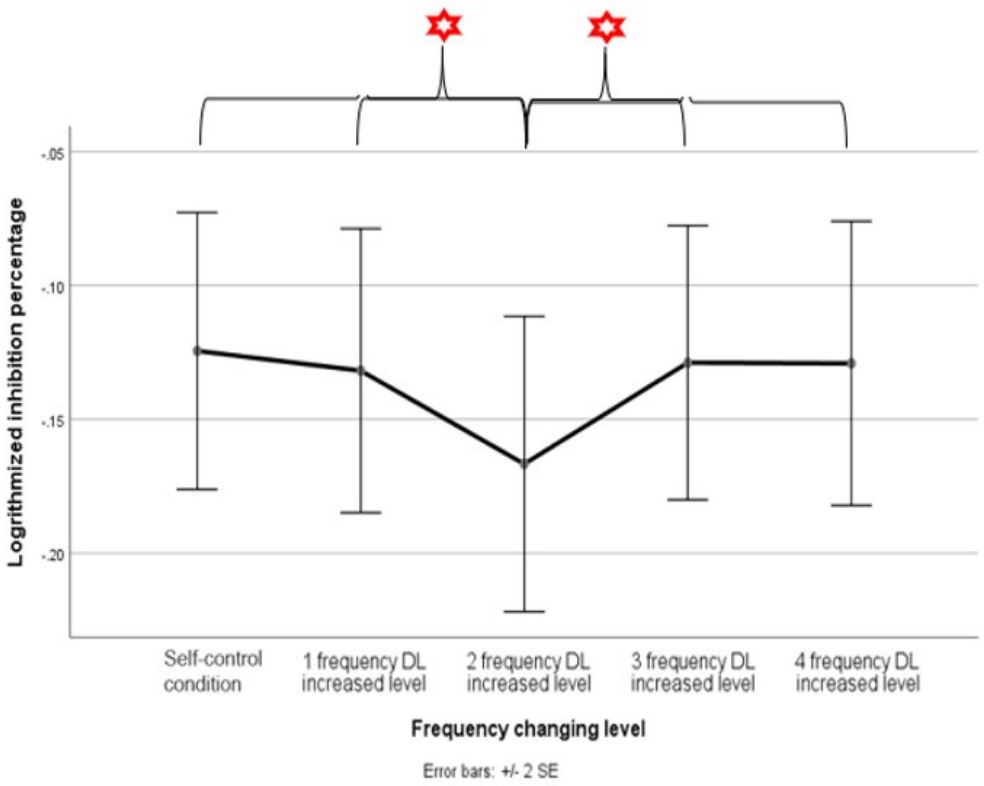
### 9.5.4.2 Frequency (Aim 2 and Aim 4 of RQ 2)

There were five parameter-changing levels in frequency (**Figure 26**): the tinnitus matched self-control condition, 1 frequency DL increased level, 2 frequency DLs increased level, 3 frequency DLs increased level, and 4 frequency DLs increased level.

**Aim2** of RQ2 was to explore if variation in frequency (See items 3, 4, 5, 6 in **Figure 26**) of the ongoing background sound leads to startle inhibition change as compared to the tinnitus

matched self-control condition (See item 2 in **Figure 26**). Part of **Aim4** was to understand within what level of variations in the frequency of the ongoing background sound, will there be no significant change in startle inhibition.

To reach Aim 2 and Aim 4, post hoc analysis was conducted using repeated measures ANOVA within the data set of frequency in the tinnitus group. The main effect of the frequency-changing level was significant, F (2.842, 437.647) =6.535, $\eta_p^2$ = 0.041, P<0.001. Pairwise comparison among levels on logarithmized inhibition percentage only showed significantly more substantial inhibition at the 2 frequency DLs increased level as compared to the self-control condition (SE=.008, p<0.001). The inhibition percentage from any of the other levels was not different from the self-control condition (Figure 42).



| Startle was NOT inhibited as compared to self-control condition | Startle was inhibited as compared to self-control condition |
|---|---|
| **1, 3 & 4 frequency DL(s) increased level** | 2 frequency DLs increased level |

**Figure 42. Logarithmized inhibition percentage across frequency changing levels. The red star indicates a statistical difference. Inhibition percentage in any gap condition = (startle magnitude of this gap condition − startle magnitude of startle only condition) / (startle magnitude of startle only condition).**

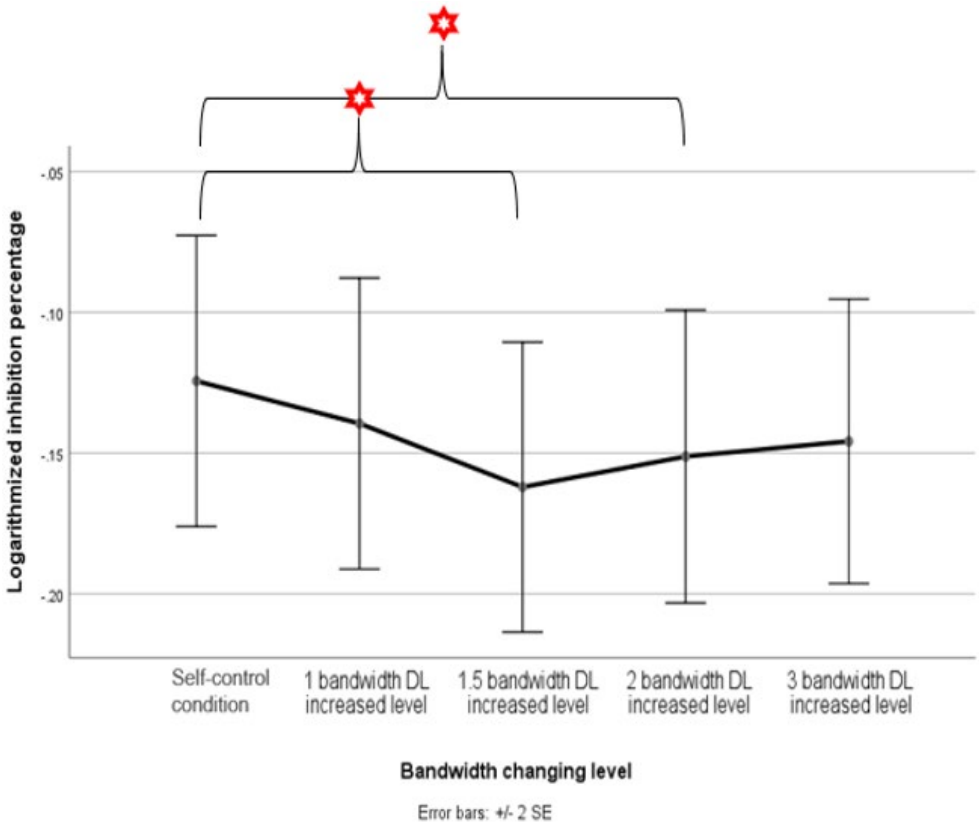
### 9.5.4.3 Bandwidth (Aim 3 and Aim 4 of RQ 2)

There were five parameter-changing levels in bandwidth (**Figure 27**): the self-control condition, 1 bandwidth DL increased level, 1.5 bandwidth DLs increased condition, 2 bandwidth DLs increased level, and 3 bandwidth DLs increased level.

**Aim3** of RQ2 was to explore if variation in frequency (See items 3, 4, 5, 6 in **Figure 27**) of the ongoing background sound leads to startle inhibition change as compared to the tinnitus matched self-control condition (See item 2 in **Figure 27**). Part of **Aim4** was to understand within what level of variations in the bandwidth of the ongoing background sound, will there be no significant change in startle inhibition.

To reach Aim 3 and Aim 4, post hoc analysis was conducted using repeated measures AVONA on logarithmized inhibition percentage within the data set of bandwidth in the tinnitus group. Based on Greenhouse-Geisser correction, the main effect of the bandwidth-changing level was significant, F (2.877, 443.059) = 5.433, $\eta_p^2$= 0.034, P=0.001. As compared to the self-control condition, pairwise comparisons on the logarithmized inhibition percentage showed significantly more startle inhibition at 1.5 bandwidth DLs of increased level, P =0.001, SE = 0.010, mean difference =0.038; at 2 bandwidth DLs of increased level, P =0.003, SE = 0.007, mean difference

=0.027. No difference on logarithmized inhibition percentage at either 3 bandwidth DLs of increased level (P =0.070, SE = 0.008, mean difference =0.021) or 1 bandwidth DL of increased level (P=0.511, SE = 0.008, mean difference =0.015) were found, as compared to the self-control condition (Figure 43).




| Startle was NOT inhibited as compared to self-control condition | Startle was inhibited as compared to self-control condition |
|---|---|
| **1 & 3 bandwidth DL(s)** | 1.5 & 2 bandwidth DLs increased level |

**Figure 43. Logarithmized inhibition percentage across bandwidth changing levels. The red star indicates a statistical difference. Inhibition percentage in any gap condition ＝ (startle magnitude of this gap condition － startle magnitude of startle only condition) ／(startle magnitude of startle only condition).**

### 9.5.5 The Third Research Question: If the Accuracy of the Match Between the Ongoing Background Sound and an Individual's Tinnitus as Defined by Self-Perception of the Match Could Predict the Gap Induced Startle Inhibition Percentage

#### 9.5.5.1 Analysis of Full Data Set and Post Hoc Analysis

The range-corrected matching scores and inhibition percentages were not normally distributed. The parameter changing level was an ordinal variable, while the range-corrected matching score was a variable measured on the scale. The level seemed to be linearly related to the range-corrected matching score on scatter plots. Based on the above three considerations, the Spearman correlation was applied to describe the degree of the relationship among the range-corrected matching score, levels, and inhibition percentage. Spearman correlation analysis indicated a strong negative association between the tinnitus range-corrected matching score and parameter changing level ($r_s$ = -0.476, p < 0.001). No other significant correlationswere found between levels, inhibition percentage, and matching score. Post hoc correlation analysis within each parameter type showed moderate associations between the range-corrected matching score and parameter changing levels within bandwidth ($r_s$ = -0.531, p < 0.001), frequency ($r_s$ = -0.336, p < 0.001) and intensity ($r_s$ = -0.590, p < 0.001). The raw matching score was also negatively associated with changing levels in each of the three parameter types (Table 26).

The raw inhibition percentage and range-corrected matching score were not correlated within bandwidth ($r_s$ = -0.040, p =0.261) and within frequency ($r_s$ = -0.017, p =0.646), but were positively correlated within intensity ($r_s$ = 0.136, p <0.001).

9.5.5.1.1Validity of Correlations

To test the validity of the above spearman's ranked correlations, the absolute value of $r_s$ was compared to the $r_s$ table derived from Zar, (1984) Table B.19. For a sample size of 31, the $r_s$ had to be larger than 0.126 to be valid (Zar, 1984). We can, therefore, conclude that the above significant correlations between the range-corrected matching score and every parameter changing level were valid since the absolute value of every $r_s$ was over 0.126. Similarly, the correlation between matching score and intensity change based on raw inhibition percentage was valid because the absolute value of $r_s$ was more than 0.126 as well. In other words, the above correlations were all valid and not found by chance (Goehring, 1981).

### 9.5.6 Startle Habituation

Six habituation trials were given to remove excessive fluctuations in startle response at the beginning of GPIAS testing. This was the same protocol used by Peterson & Blumenthal, 2018). However, habituation of startle response was still observed across trials in each parameter, with slightly different habituation among parameters. Specifically, habituation of the startle occurred after the 1st trial of intensity change related testing conditions (Figure 44, Panel A), while habituation occurred after the 2nd trial in frequency change ( Figure 44, Panel B) or bandwidth change (Figure 44, Panel C) related testing conditions.

Short-term habituation is traditionally defined as an attenuation of the startle response on repeated presentation of the startle stimuli within a session that is reversible quickly. The reversion for a short-term habituation could be within a few minutes in rats (Pilz & Schnitzler, 1996), but

was less clear in humans. Based on the available evidence, the short-term habituation could be reversed in healthy human in at least two hours (Abel et al., 1998). In the above definition of short-term habituation, a session is the same as a specific parameter changing level in the current study. In the current study, four trials were lined up and played one after another within each parameter changing level. Data from the current study imply a short-term habituation seems reversible within less than one minute (playing four trials took less than one minute) when the reversion was induced by parameter change in the ongoing background sound of a GPIAS test. Such parameter change could be a change from one type of parameter to another type, and also could be a change from one level to another level within the same parameter. Nevertheless, this type of super-short-term habituation did not affect the statistical analysis applied to the research questions, since the inhibition percentage was always calculated based on the averaged startle magnitude from the four trials.

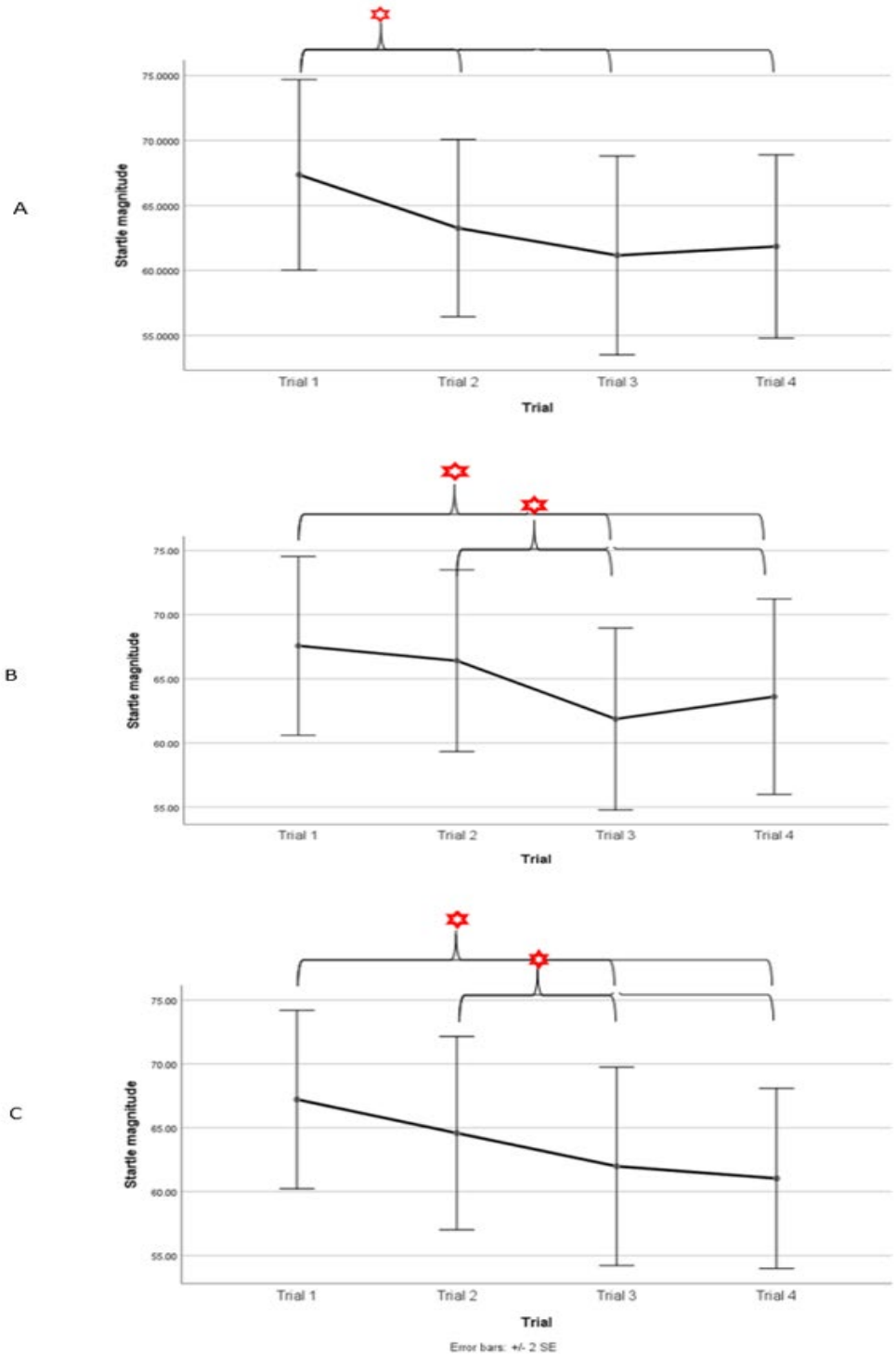



**Figure 44. Habituation of startle responses across trials. Panel A: intensity change related startle magnitudes decrease across trials. Panel B: frequency change related startle magnitudes decrease across trials. Panel C: bandwidth change related startle magnitudes decrease across trials.**

## 9.6 Discussion

### 9.6.1 The Primary Research Questions: If Tinnitus Filling in the Gap Was the Cause for the Lack of Inhibition in Individuals with Continuous Tonal Tinnitus as Compared to Individuals without Tinnitus

Tinnitus filling in the gap was an important assumed theory to be tested before answering the second research question, which was to explore the amount of deviation in frequency, intensity, or bandwidth in the ongoing background sound created from the tinnitus match required to obtain a startle inhibition as compared to the tinnitus matched self-control condition (no inhibition). This assumed theory was tested as the primary research question, which asked if tinnitus filling in the gap was the cause for a lack of inhibition in people with tinnitus as compared to people without tinnitus. For the primary research question, it was assumed that for individuals with no tinnitus, a startle inhibition would be measured when there was a silent gap presented (self-control condition) in the GPIAS paradigm as compared to when the ongoing background sound was played without a gap presented (startle-only condition). It was also assumed that for individuals with tinnitus, there would be no startle inhibition in the self-control condition (silent gap presented with the assumed theory that tinnitus is filling in the gap). In other words, no difference in startle inhibition between the startle-only condition and the self-control condition for individuals with tinnitus where the background sound was matched to their self-reported tinnitus perception.

Since the results showed that there was no inhibition in the self-control condition within the tinnitus group, but there was inhibition in the same condition within the no-tinnitus group, we were able to conclude that tinnitus filled in the gap of the self-control condition in the tinnitus group. However, one might be concerned that the loudness tolerance level difference between groups could potentially temper this conclusion. Although subjects with clinically significant hyperacusis were excluded from the study, as a group, the individuals reporting tinnitus had lower (quieter levels) thresholds of discomfort compared to the no-tinnitus group. Although hyperacusis might indirectly affect the startle magnitude through the aversive emotional reaction (Tyler et al., 2014; Vaidyanathan, Patrick, & Bernat, 2009), there is no evidence that hyperacusis or relatively low tolerance levels of loudness would affect the inhibition percentage which was used as the measurement of interest in this study. Regardless, further analysis was conducted to address this potential concern. Due to an unbalanced sample size between groups, linear mixed effects models (LMM) was used as an analytical method. In this LMM analysis, averaged LDL across frequencies of each ear were included as the covariates, logarithmized inhibition percentage in the self-control condition was the dependent variable, and the group was the independent variable. The 1$^{st}$ round of model fitting results revealed that the averaged LDL of the right ear ($F=0.878$, $p=0.442$) and left ear ($F=0.358$, $P=0.550$) were both non-significant co-variates, and therefore, were removed for the 2$^{nd}$ round of model fitting. Removing the two co-variables from the model led to a decrease in Akaike's Information Criterion (AIC) from 134.888 to 131.080, and a decrease in Schwarz's Bayesian Criterion (BIC) from 147.980 to150.718, indicating the 2$^{nd}$ round had a better model fit compared to the 1$^{st}$ one. In the 2$^{nd}$ model, there was a significant difference between groups, $F(1,195)=5.505$, $p=0.020$. Pairwise comparison between groups showed a smaller logarithmized startle inhibition percentage in the tinnitus group as compared to no-tinnitus group (Mean

Difference= 0.139), which still implied a lack of inhibition in participants with tinnitus and matched with analysis results from Mann-Whitney U test.

### 9.6.2 The Second Research Questions: If Deviations in Intensity, Frequency, or Bandwidth of the Ongoing Background Sound as Compared to a Background Sound Maximally Matched to an Individual's Tinnitus Perception Could Induce Startle Inhibition

#### 9.6.2.1 Intensity Change Induced Startle Inhibition

The second research question (RQ2) asked whether deviations in parameters of the ongoing background sound as compared to the tinnitus maximally matched background sound could induce startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation in parameters would not lead to startle inhibition. Specifically, **Aim1** of RQ2 was to explore if variation in intensity (See items 3, 4, 5, 6 in **Figure 25**) of the ongoing background sound leads to startle inhibition change as compared to the tinnitus matched self-control condition (See item 2 in **Figure 25**). Part of **Aim4** was to understand within what level of variations in the intensity of the ongoing background sound, will there be no significant change in startle inhibition.

The null hypothesis (H0) for Aim 1 of RQ2 was rejected because the variation of the ongoing background sound led to startle inhibition change. The H1 for Aim 1 predicted that variation in the intensity of the ongoing background sound would lead to startle inhibition change, and a larger magnitude of variations in intensity would lead to a more significant inhibition change as compared to the inhibition percentage at the self-control condition. The results, therefore, only partially supported H1 for Aim1, since although we observed that variation in the intensity of the ongoing background sound led to startle inhibition change, an

increase in the magnitude of variations in intensity did not always lead to a larger inhibition change as compared to the inhibition percentage at the self-control condition.

Concerning Aim 4 of RQ2, pairwise comparisons between levels showed gradually increased inhibition occurred after stepwise increasing the intensity of background sound compared to the self-control condition. Still, such further inhibition was not significantly more extensive compared to the self-control condition until reaching three intensity DLs increased level and four intensity DLs increased level. From the graph and pairwise comparisons, we could tell that there was a generally linear increase in inhibition induced by gradually increasing the intensity contrast between background sound and perceived tinnitus (Figure 41) until reaching 3 intensity DL increase level. This trend of linear increase in inhibition was matched with findings from a similar human GPIAS study from Peterson and Blumenthal (2018) and human SPIAS study from Swerdlow et al. (2007) (Figure 45). Higher intensity of sound-prepulse induces more substantial inhibition of startle when the intensity of prepulse was < 16 dB SPL over a background of 70 dB SPL white noise in rats (Swerdlow et al., 2005, 2002). There was a similar linear ascending manner in terms of the intensity function of gap-prepulse < 15 dB SPL over a background of 65 dB SPL white noise in normal-hearing humans (Peterson & Blumenthal, 2018). Although the previous human GPIAS study failed to provide us with information on startle inhibition after 20 dB of contrast between the background sound and the gap-filling sound, the SPIAS study from Swerdlow et al. (2007) showed that a gradual increase in intensity contrast after 20 dB resulted in an incremental decrease in inhibition (Figure 45), which indicated a bimodal linear pattern in inhibition change induced by increasing the intensity in the background sound. Of note, since the averaged intensity DL of the qualified 31 participants with tinnitus in the current study was around 4 dB, but with a range between 1 dB as the minimal and 8 dB as the maximal intensity DL, a

turning point of the bimodal linear pattern at three intensity DLs increased level would be more or less matched with results from these previous studies.

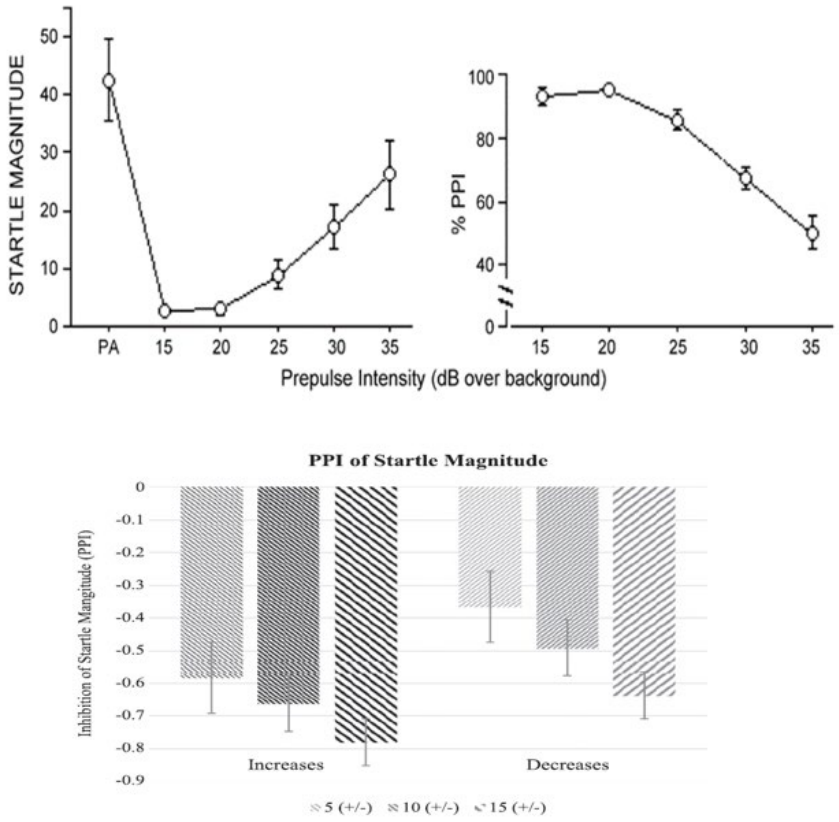



**Figure 45. Effects of prepulse intensity on PPI. Top left figure: startle magnitude and. Top right figure: %PPI as a function of prepulse intensity for 13 startles ‘‘responders’’ in this session. Error bars: standard error. Bottom figure: Stimulus intensity increases as compared to background sound are depicted in black (left), and stimulus intensity decreases as compared to background sound are depicted in gray (right). Greater change from background resulted in more significant magnitude inhibition. (Top two figures are from Swerdlow et al. (2007) with permission. Bottom figure is from Peterson and Blumenthal (2018) with permission.)**

#### 9.6.2.2 Frequency Change Induced Startle Inhibition

The second research question (RQ2) asked whether deviations in parameters of the ongoing background sound as compared to the tinnitus maximally matched background sound could induce startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation in parameters would not lead to startle inhibition. Specifically, **Aim2** of RQ2 was to explore if variation in frequency (See items 3, 4, 5, 6 in **Figure 26**) of the ongoing background sound leads to startle inhibition change as compared to the tinnitus matched self-control condition (See item 2 in **Figure 26**). Part of **Aim4** was to understand within what level of variations in the frequency of the ongoing background sound, would there be no significant change in startle inhibition.

The null hypothesis (H0) for Aim 2 of RQ2 was rejected. H1 suggested that variation in the frequency of the ongoing background sound would lead to startle inhibition change, and an increase in the magnitude of variations in frequency would lead to a more substantial inhibition change as compared to the inhibition percentage of the self-control condition. The results only partially supported H1 since an increase in the magnitude of variations in frequency did not always lead to a larger inhibition change as compared to the inhibition percentage of the self-control condition. However, we did observe that some variations in the frequency of the ongoing background sound led to startle inhibition change. This frequency change based startle inhibition phenomenon mirrors a similar effect of a frequency shift on startle inhibition reported in previous human studies and animal studies with rats (Cranney, Cohen, & Hoffman, 1985; Marsh, Hoffman, Stitt, & Schwartz, 1975; Peterson & Blumenthal, 2018).

#### 9.6.2.2.1 The Notch Pattern of Frequency Change Induced Inhibitions

Related to Aim 4 of RQ2, the 2 frequency DLs increased level induced significantly more inhibition compared to other levels, including the self-control condition, which makes the pattern of results look like a notch exists at the 2 frequency DLs increased level. PreciselyThe self-control condition induced 0.042 less logarithmized inhibition percentage compared to 2 frequency DLs increased level, SE=.008, $p<0.001$; the 1 frequency DL increased level induced 0.035 less logarithmized inhibition percentage, SE=0.009, $p=0.001$; the 3 frequency DLs increased level induced 0.038 less logarithmized inhibition percentage, SE=0.008, $P<0.001$; and the 4 frequency DLs increased level induced 0.038 less logarithmized inhibition percentage, SE=0.013, $P=0.042$. No other between-level comparisons were significant (Table 26).

Stitt et al., (1974) found that when a ½ octave shift in an otherwise steady band of noise occurred 64 msec before an intense (startle-eliciting) burst of noise, the amplitude of the startle reaction in rats was reduced. After Stitt's study, Marsh et al. found frequency shifts as small as 1/16 octave were able to inhibit the startle significantly in rats (Marsh et al., 1975). In our participants with tinnitus, the average central frequency of tinnitus was 5802.5 Hz, of which the 1/16 octave of frequency shift would be 362.7 Hz. However, the averaged frequency DL of our tinnitus group was 268.2 Hz, which was, therefore, slightly smaller than the 1/16 octave of frequency shift found in rats. Since there was a statistically significant inhibition at 2 frequency DLs condition in the current study, but not at 1 frequency DL condition, the current finding from humans was in line with what was reported by Marsh et al., (1975) in rats in terms of the smallest frequency shift that had the capability of inhibiting a startle response.

The frequency change induced inhibition pattern looks similar to what was found from the data set of bandwidth, that there was a notch at 2 frequency DLs increased level (Figure 45). Cranney's (1985) animal study could potentially explain the notch (Cranney et al., 1985). As an indirect comparison, Cranney et al. (1985) plotted an inhibition curve that showed a small "notch" at 2.5 kHz of frequency shift, while we plotted an inhibition curve that showed a small "notch" at 2 frequency DLs of shifted level (Figure 42). Although Cranney's study was using naïve albino rats as testing subjects and was limited to relatively small sample size (N=14), the plots from Cranney's (1985) article still indicated a trend that was similar to the current study result. Cranney (1985) found that a frequency shift added to the amount of inhibition only when the gap duration was relatively short (10 ms), while a longer gap duration (100 ms) appeared to produce maximum inhibition. However, the frequency shift did not have any statistically significant effect, which was likely a result of a small sample size. However, the plots (Figure 46) from Cranney's study still indicated a trend to inhibit more at the frequency shift around 2.50 kHz when compared to the gap-only (no frequency shift) condition, and their plots also showed less inhibition occurred when the frequency shift was either smaller or larger than 2.50 kHz.

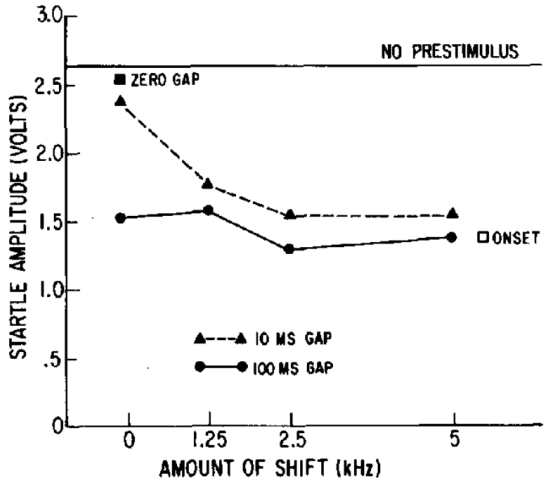



**Figure 46. Mean amplitude of startle response (across subjects) as a function of the amount of frequency shift and the gap duration. (The horizontal line indicates the mean amplitude of startle response elicited by the noise burst without prior acoustic stimulation.) With permission from Cranney et al. 1985.**

Of note, there was no 3.75 kHz data point between the 2.50 kHz frequency shift level and 5 kHz frequency shift level in the study from Cranney et al., (1985), so the loss of impact from frequency shift on startle inhibition might show up earlier than the 5 kHz frequency shift level. From our current study, the loss of impact of frequency shift (from the background sound to the tinnitus in the gap) occurred right after 2 frequency DLs level, but no sign of any frequency shift impact as to the startle inhibition before 2 frequency DLs level. In other words, the inhibition occurred right at 2 frequency DLs increased level only.

Other than comparing with previous study results, one might suggest that the audibility of the background sound might play a role in explaining the notch in the pattern of frequency change induced inhibitions. We can examine the data by comparing it to the equal-loudness contour. The hypothesis was that based on the equal-loudness contour (Fletcher & Munson, 1933), the audibility of the ongoing background sound would increase as the central frequency of the background sound was closer to 4 kHz. In contrast, the audibility of the background sound would decrease as the

frequency of background sound was away from 4 kHz. However, this hypothesis was not supported by the current data by comparing the frequency changed induced inhibition pattern from a sub-group of participants considered to have low-frequency tinnitus (<4 kHz) compared to a sub-group with high-frequency tinnitus (> 4 kHz).

Except for limited evidence from one animal study, there is no theory or additional evidence that would explain why the startle inhibition did not maintain or become even more pronounced when the frequency of the background sound deviated sufficiently (>2 DLs) from the frequency of the tinnitus perception.

#### 9.6.2.2.2 Frequency Contrast Causes the Inhibition: Additional Evidence of Tinnitus Filling in the Gap

It demonstrated in the earlier sections of this manuscript that tinnitus filling in the gap was the reason for lack of inhibition at the self-control condition in the tinnitus group. However, unlike the lack of inhibition at self-control condition, when the background sound's central frequency-shifted 2 DL higher than the tinnitus's central frequency, the startle was inhibited in participants with tinnitus. Therefore, there must be another factor other than the gap itself that causes that substantial inhibition. What was the other factor that led to startle inhibition? In sound prepulse inhibition of acoustic startle (SPIAS) studies, Basavaraj and Yan (2012) and Stitt et al. (1974) suggested that shifts in the frequency spectrum of constant noise could inhibit startle magnitude due to the frequency difference between the sound prepulse and background sound rather than the frequency of the background sound itself. Similarly, in a GPIAS study from Cranney et al., (1985), having a gap and a frequency shift occurring right after the gap (Figure 47) induced more inhibition than what a gap alone or a frequency shift alone could induce.

Taken together, it is reasonable to infer that various frequency contrasts between the continuous tonal tinnitus and the ongoing background sound were the driver for the change in startle inhibition percentage, which adds to evidence that further supports the theory of tinnitus filling in the gap and answers the primary research question (*if tinnitus filling in the gap of a GPIAS testing paradigm caused the lack of startle inhibition in individuals with tinnitus as compared to individuals without tinnitus*).

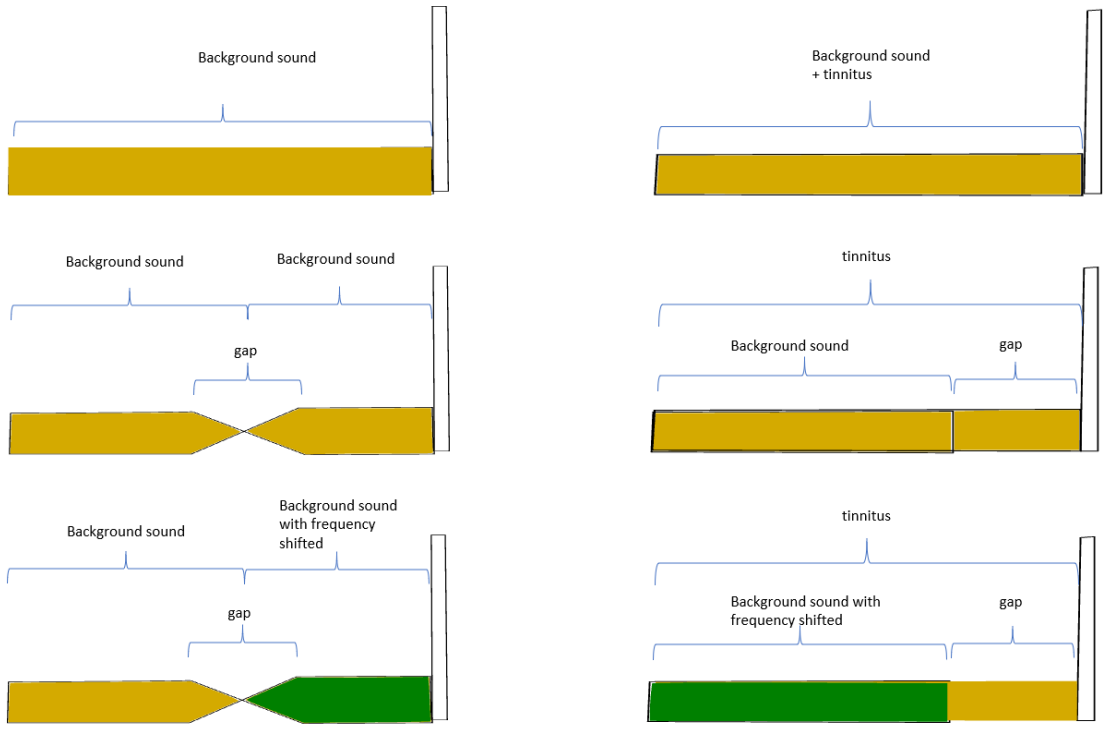



**Figure 47. Illustration of frequency shifting conditions from two studies. The left three conditions are from Cranney et al., 1985, and the right three conditions are from the current study.**

### 9.6.2.3 Bandwidth Induced Startle Inhibition

The second research question (RQ2) asked whether deviations in parameters of the ongoing background sound as compared to the tinnitus maximally matched background sound could induce a startle inhibition change in the GPIAS testing paradigm. If so, what range of deviation in parameters would not lead to startle inhibition. Specifically, **Aim3** of RQ2 was to explore if variation in bandwidth (See items 3, 4, 5, 6 in **Figure 28**) of the ongoing background sound leads to startle inhibition change as compared to the tinnitus matched self-control condition (See item 2 in **Figure 28**). Part of **Aim4** was to understand within what level of variations in the bandwidth of ongoing background sound, will there be no significant change in startle inhibition.

The null hypothesis (H0) for Aim 3 of RQ2 was rejected. H1 suggested that variation in the bandwidth of ongoing background sound would lead to startle inhibition change, and an increase in the magnitude of variations in bandwidth could lead to a more substantial startle inhibition change as compared to the startle inhibition percentage at the self-control condition. The results only partially supported the H1 since an increase in the magnitude of variations in bandwidth did not always lead to a larger inhibition change as compared to the inhibition percentage at the self-control condition.

Based on the averaged logarithmized inhibition percentage across conditions, a bimodal linear change was found (Figure 43). Specifically, a linear increase in inhibition was noticed at, and before 1.5 bandwidth DLs increased level, possibly benefiting from an unmatched contrast between the background sound and the perceived tinnitus. Still, a linear decrease in inhibition was noticed at and after 2 bandwidth DLs increased level, a likely result of a categorical change from a tonal sound to a broadband noise sound, which was found in previous SPIAS studies that showed more significant prepulse inhibition of perceived stimulus intensity with broadband noise prepulse than with pure tone prepulse (Swerdlow et al., 2007; Wynn, Dawson, & Schell, 2000). In the study from Swerdlow et al., (2007), however, such categorical difference induced inhibition was not existent when the sound prepulse was longer than 50 ms (Figure 48). Still, in our study, a more extensive inhibition was observed when the background sound was a broadband noise (i.e., at and after 2 bandwidth DLs increased), even though the gap prepulse was 120 ms. Swerdlow's study was a SPIAS study, while the current one was a GPIAS study. With the same amount of contrast between prepulse and the background sound, SPIAS inhibits more than GPIAS does. The current study finding implies that a gap if embedded in a continuous broadband noise inhibits startle response less than a gap if embedded in a continuous narrowband sound when the gap is 120 ms

long and located right before a startle stimulus. No other statistical differences were found (Appendix: Table 22).

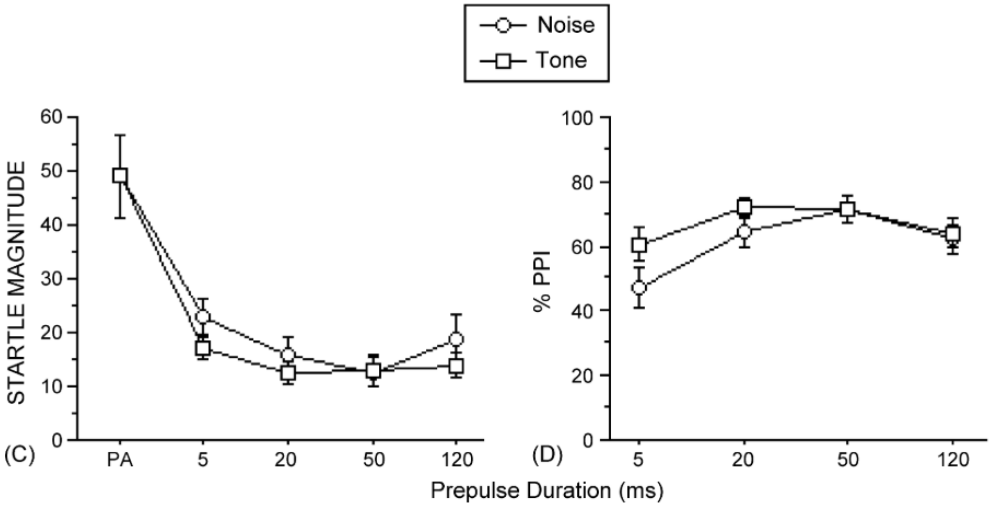



**Figure 48. Illustration of noise prepulse and tone prepulse induced prepulse inhibition as a function of prepulse duration. With permission from Swerdlow et al., 2007.**

### 9.6.3 The Third Research Question: If the Accuracy of the Match Between the Ongoing Background Sound and an Individual's Tinnitus as Defined by Self-Perception of the Match Could Predict the Gap Induced Startle Inhibition Percentage

The third research question (**RQ3**) was whether the accuracy of matching between tinnitus and the ongoing background sound in the GPIAS paradigm, as defined by the self-perception of a match, could predict the gap induced startle inhibition percentage. The magnitude of startle response was measured with ocular EMG. The specific aim (**Aim 1**) of RQ3 was to see if the higher self-reported ratings of tinnitus matching accuracy were associated with less gap induced

startle inhibition when these signals were used for the ongoing background sound (See Figure 28). A correlation analysis was performed to answer RQ3 and the related aim.

All significant correlations found based on the current data were valid and not found by chance based on validity test results. Specifically, a larger parameter change in the background sound could powerfully predict a lower self-perceived matching score in participants with continuous tonal tinnitus from the current study. This parameter change could be any of the three targeted parameters, including bandwidth, frequency, and intensity (Figure 49), implying that participants with tinnitus were able to identify the amount of difference between the external stimuli and their perceived tinnitus when the difference was caused by the change on any of the three parameters. In other words, people with tinnitus could correctly tell experimenters how well or poorly the external sound (i.e., background sound) was matched to their tinnitus.

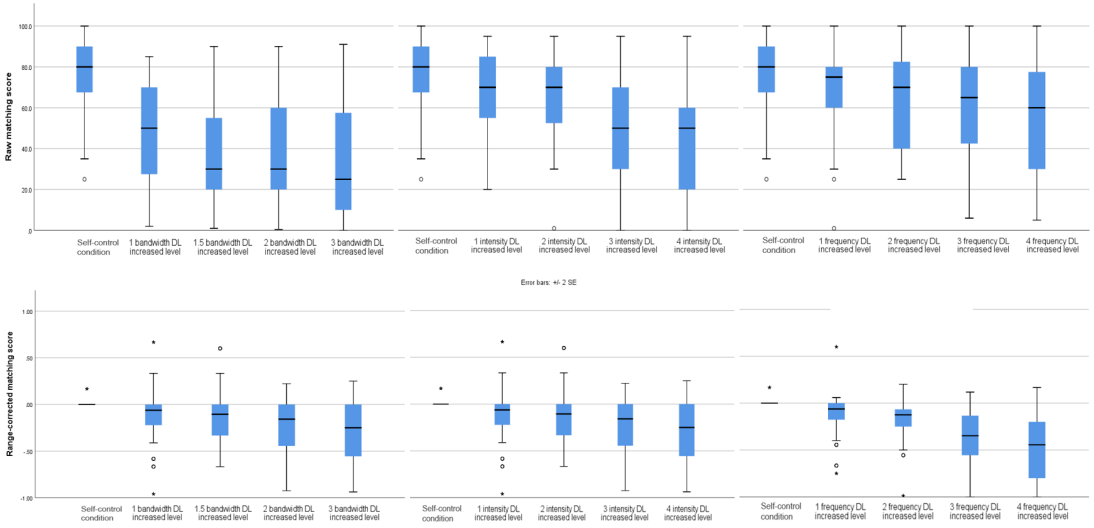



**Figure 49. Boxplot of the raw matching score (top panel) and range-corrected matching score (bottom panel) by parameter changing level.**

Correlation results also indicated an intensity-change induced lower matching score was associated with a more significant inhibition percentage (Figure 50). Nevertheless, a lower range-corrected matching score might not be used to predict a more extensive inhibition, since the strength of correlation was very weak. Additionally, since such association was not existent by changing the bandwidth or frequency of the background sound, the conclusion could be drawn that self-perceived matching score between tinnitus perception and the ongoing background sound in GPIAS testing paradigm should not be used to predict the amount of startle inhibition in this paradigm.

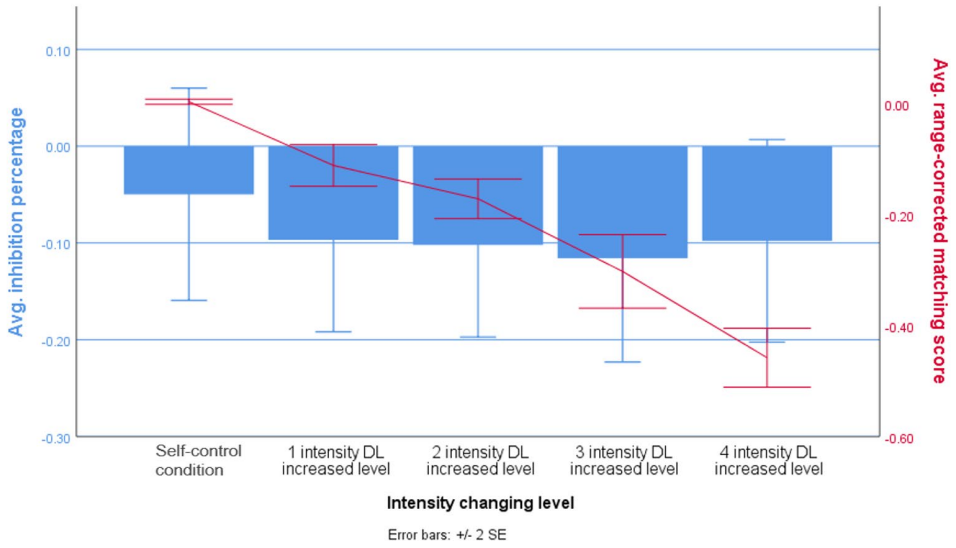



**Figure 50. Inhibition percentage and range-corrected matching score as functions of intensity changing level.**

### 9.6.4 Study Implication and Future Direction

Beyond the direct framework of the study, this research provides tightly connected research implications. First, the present study has made a further step testing the theory of tinnitus filling in the gap. Creating a background sound that closely matched each individual's tinnitus allowed a controlled method for testing the assumed theory that tinnitus fills in the silent gap (in the self-control condition) mimicking the startle-only condition (when the background sound continues throughout the gap) and no startle inhibition is revealed. Further, particular levels of parameter change in the background sound that generate a contrast between the background sound and tinnitus perception at the gap worked as a prepulse to inhibit the startle response. The results indicated when the amount of intensity, frequency, and bandwidth contrast between the background sound and each individual's tinnitus was within 1 DL, there was no significant difference in terms of startle inhibition. This range of matches where results do not change may be of interest to animal researchers since the GPIAS paradigm has been widely utilized as an assessment for tinnitus in animals where it is almost impossible to match the external testing sound with the animal's tinnitus exactly. The current study verified that even with careful matching along three parameters, a perfect match is not achieved, and participants are able to quantify the difference between their tinnitus and the background sound, as reflected by the self-perceived matching rating score (Figure 49). Therefore, clinical audiologists or researchers may adopt self-rating more confidently as a way to check if and how well the sound is nicely matched when patients with tinnitus are using patient-driven tinnitus parameter matching procedures.

Based on findings from this current study, future research can be focused on exploring neural circuits of GPIAS. While the neural circuits of SPIAS have been well studied, the neural

circuits of GPIAS remain unclear. What is known to us is that although both SPIAS and GPIAS share part of a common startle neural circuitry, the inhibition of the acoustic startle response by prepulse or gap is orchestrated by different neural pathways. For instance, sound prepulse is activating the lateral globus pallidus (LGP) to inhibit the startle response, while gaps bypass the LGP to activate the auditory cortex (Moreno-Paublete et al., 2017). Additionally, although little is known, it is plausible that subregions within the shared structures of the brainstem might respond differently to a gap prepulse and a sound prepulse. Exploring acoustic functions that can affect the startle response of SPIAS and GPIAS differently will help further identify the involved sub-structures within the brainstem. One approach to unraveling the separate neural structures of SPIAS from GPIAS is to explore whether some of the acoustic phenomena that had been observed in SPIAS studies could be replicated in GPIAS studies to some extent. For example, the particular level of intensity contrast between background sound and sound prepulse could lead to a consistently more substantial startle inhibition compared to the inhibition induced by the same amount of intensity contrast between the background sound and the embedded gap prepulse. This would indicate that inhibition of the acoustic startle response by sound-prepulse or gap-prepulse is arranged by different neural pathways.

Alternatively, by finding acoustic factors that modulate only SPIAS or GPIAS, but not both of them, the divided neural structures amidst the generally overlapped startle neural circuitry might be further disentangled. For instance, the interaural disparities of binaural sound prepulse inputs used for sound localization were found not capable of modulating the startle reflex in SPIAS study from Li & Frost (2000). This phenomenon was called the omnidirectionality of startle inhibition. The IC-SC-PPTg-PnC connection (Figure 51) was proposed by the authors as the pathway to explain this phenomenon in SPIAS paradigm because, in mammals, the spatial laterality

information of Inferior Colliculus (IC) on one side of an individual can be projected only ipsilaterally to Superior Colliculus (SC) which is on the same side of IC. This laterality information at SC is then projected bilaterally to the pedunculopontine tegmental nucleus (PPTg) on both sides. Each side of PPTg further projects bilaterally to the caudal pontine reticular nucleus (PnC). Of note, one side of the PnC receives bilateral projections from two PPTgs and, therefore, is affected by laterality information from two ICs (Figure 51). Future studies can be focused on verifying whether such omnidirectionality of prepulse inhibition from SPIAS is also existent using the GPIAS paradigm, which might help tease out if the GPIAS and SPIAS paradigms share the IC-SC-PPTg-PnC connection.

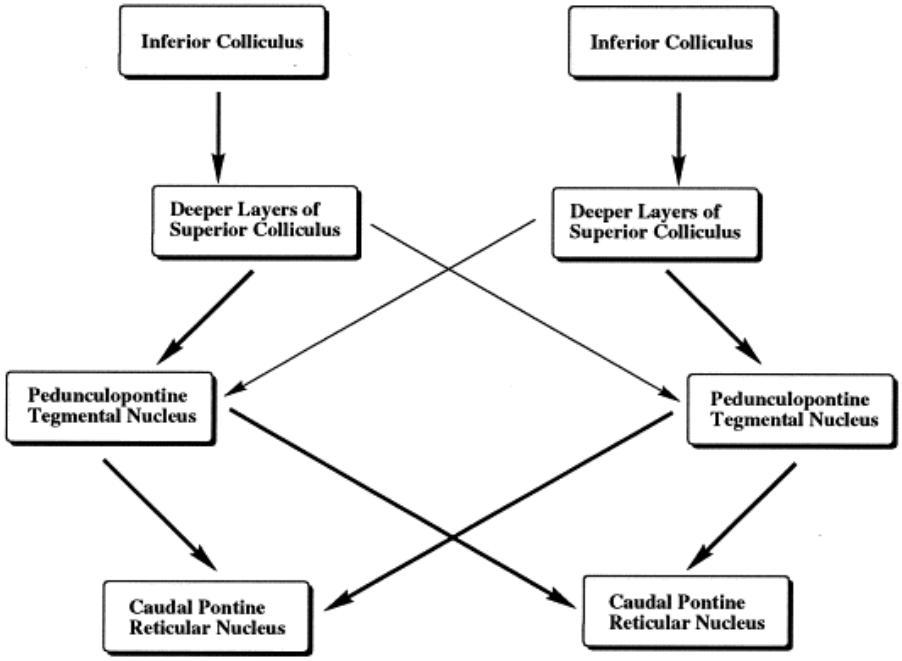



**Figure 51. Schematic diagram showing the hypothetical neural pathways that mediate acoustic prepulse inhibition by linking the inferior colliculus and the caudal pontine reticular nucleus (with permission from Li & Frost, 2000).**

### 9.6.5 Study Limitations

Several limitations should be considered when evaluating the current results. The study is limited to participants with continuous tonal tinnitus, who also had normal hearing thresholds, without clinically defined hyperacusis and medication/disease history that would potentially affect the acoustic startle response. The study conclusions cannot be generalized to certain situations since only the DL of increased conditions rather than the decreased conditions were included to avoid a potential directional effect. Certain situations including but not limited to when the background sound is getting gradually quieter, lower-pitched than tinnitus, or both were not examined.

### 9.6.6 Conclusion

Continuous tonal tinnitus constitutes an inability to hear silence, and hence fills a silent gap in a GPIAS test, which affects the startle inhibition percentage in people with this type of tinnitus as compared to people without tinnitus. However, having tinnitus or not is not the only critical factor that affects the startle inhibition percentage. This study confirmed that certain levels of parameter mismatches between the GPIAS background sound and continuous tonal tinnitus could bring changes to the startle inhibition percentage. Specifically, no startle inhibition in GPIAS testing can be expected if the continuous tonal tinnitus deviants from the ongoing background sound in no more than 1 frequency DL, 1 bandwidth DL, or 2 intensity DLs. If above this range, there could be a difference.

In short, the current study found that GPIAS paradigm exclusively tests continuous tonal tinnitus, only when confounding factors are well controlled. The measured inhibition deficits in

individuals with tinnitus are due to tinnitus filling in the gap, and other factors. Any parameter deviant between ongoing background sound and continuous tonal tinnitus is one of the factors that can affect the inhibition percentage.

## Appendix A : Tinnitus Sample Case History Questionnaire (TSCHQ)

Tinnitus sample case history questionnaire (TSCHQ) developed by Tinnitus Research Initiative, UK:

https://www.tinnitusresearch.net/images/files/migrated/consensusdocuments/en/TINNITUS_SAMPLE_CASE_HISTORY_QUESTIONNAIRE.pdf

"Items list" for tinnitus case history questionnaires:

https://docs.google.com/viewer?url=https%3A%2F%2Fwww.tinnitusresearch.net%2Fimages%2Ffiles%2Fmigrated%2Fconsensusdocuments%2Fen%2FItems-list.pdf

**In the current study, subjects only answer questions in the category "A" (=essential) in TSCHQ, which end up with 14 questions.**

# Appendix B : Randomization Order of Ocular EMG

**Table 21. Randomization order for the tinnitus group**

| ID # | habituation trial | startle only trial | original gap trial | 1 bdw DL increased | 1.5 bdw DL increased | 2 bdw DL increased | 3 bdw DL increased | 1 freq DL increased | 2 freq DL increased | 3 freq DL increased | 4 freq DL increased | 1 inten DL increased | 2 inten DL increased | 3 inten DL increased | 4 inten DL increased |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 8 | 5 | 13 | 14 | 3 | 12 | 2 | 6 | 10 | 4 | 1 | 9 | 11 | 7 |
| 2 | 0 | 9 | 7 | 14 | 3 | 1 | 12 | 13 | 4 | 5 | 11 | 10 | 6 | 8 | 2 |
| 3 | 0 | 11 | 6 | 8 | 13 | 14 | 1 | 9 | 5 | 12 | 4 | 3 | 10 | 7 | 2 |
| 4 | 0 | 4 | 13 | 14 | 5 | 12 | 3 | 2 | 6 | 9 | 7 | 8 | 11 | 1 | 10 |
| 5 | 0 | 3 | 12 | 2 | 7 | 10 | 1 | 11 | 8 | 13 | 4 | 5 | 9 | 14 | 6 |
| 6 | 0 | 13 | 7 | 4 | 8 | 14 | 3 | 11 | 12 | 6 | 1 | 10 | 5 | 9 | 2 |
| 7 | 0 | 7 | 14 | 6 | 5 | 10 | 1 | 3 | 8 | 13 | 9 | 4 | 11 | 2 | 12 |
| 8 | 0 | 7 | 8 | 9 | 1 | 3 | 4 | 2 | 10 | 14 | 13 | 12 | 5 | 6 | 11 |
| 9 | 0 | 7 | 12 | 1 | 14 | 13 | 10 | 4 | 11 | 6 | 2 | 9 | 5 | 3 | 8 |
| 10 | 0 | 6 | 11 | 5 | 7 | 3 | 2 | 12 | 4 | 13 | 14 | 8 | 10 | 1 | 9 |
| 11 | 0 | 1 | 7 | 4 | 8 | 10 | 6 | 13 | 3 | 5 | 14 | 12 | 2 | 9 | 11 |
| 12 | 0 | 9 | 11 | 13 | 12 | 10 | 14 | 5 | 4 | 2 | 3 | 1 | 8 | 6 | 7 |
| 13 | 0 | 4 | 1 | 7 | 14 | 5 | 9 | 13 | 12 | 6 | 11 | 2 | 3 | 8 | 10 |
| 14 | 0 | 13 | 7 | 6 | 14 | 9 | 5 | 12 | 11 | 2 | 8 | 3 | 1 | 10 | 4 |
| 15 | 0 | 1 | 14 | 8 | 2 | 7 | 13 | 9 | 11 | 5 | 12 | 4 | 3 | 6 | 10 |
| 16 | 0 | 7 | 14 | 9 | 6 | 8 | 12 | 10 | 3 | 5 | 1 | 11 | 13 | 2 | 4 |
| 17 | 0 | 8 | 9 | 4 | 13 | 3 | 14 | 5 | 2 | 12 | 7 | 10 | 11 | 1 | 6 |
| 18 | 0 | 10 | 3 | 11 | 1 | 6 | 14 | 7 | 4 | 5 | 8 | 2 | 9 | 12 | 13 |
| 19 | 0 | 13 | 11 | 6 | 7 | 2 | 9 | 12 | 8 | 4 | 1 | 10 | 3 | 14 | 5 |
| 20 | 0 | 6 | 4 | 14 | 7 | 2 | 13 | 8 | 5 | 3 | 11 | 9 | 10 | 12 | 1 |
| 21 | 0 | 8 | 9 | 3 | 6 | 5 | 2 | 10 | 4 | 12 | 14 | 11 | 1 | 13 | 7 |
| 22 | 0 | 7 | 9 | 13 | 8 | 1 | 3 | 2 | 14 | 5 | 11 | 10 | 12 | 4 | 6 |
| 23 | 0 | 7 | 1 | 4 | 5 | 10 | 14 | 11 | 8 | 9 | 3 | 6 | 2 | 13 | 12 |
| 24 | 0 | 4 | 3 | 5 | 13 | 1 | 6 | 12 | 9 | 10 | 2 | 7 | 11 | 14 | 8 |
| 25 | 0 | 3 | 13 | 4 | 2 | 7 | 8 | 9 | 1 | 12 | 11 | 14 | 10 | 6 | 5 |
| 26 | 0 | 7 | 13 | 9 | 11 | 8 | 3 | 5 | 4 | 10 | 2 | 1 | 14 | 12 | 6 |
| 27 | 0 | 4 | 1 | 14 | 9 | 13 | 2 | 6 | 11 | 10 | 3 | 8 | 5 | 7 | 12 |
| 28 | 0 | 1 | 13 | 5 | 4 | 6 | 10 | 11 | 2 | 7 | 3 | 8 | 14 | 9 | 12 |
| 29 | 0 | 5 | 7 | 1 | 10 | 11 | 3 | 12 | 9 | 6 | 4 | 14 | 8 | 13 | 2 |
| 30 | 0 | 4 | 8 | 5 | 7 | 11 | 3 | 13 | 1 | 6 | 2 | 12 | 9 | 14 | 10 |
| 31 | 0 | 1 | 2 | 7 | 12 | 10 | 14 | 3 | 8 | 11 | 13 | 9 | 6 | 5 | 4 |
| 32 | 0 | 5 | 14 | 11 | 1 | 4 | 3 | 13 | 8 | 6 | 7 | 12 | 9 | 10 | 2 |
| 33 | 0 | 1 | 5 | 8 | 11 | 3 | 4 | 14 | 10 | 2 | 7 | 6 | 13 | 9 | 12 |
| 34 | 0 | 10 | 5 | 11 | 12 | 2 | 4 | 14 | 9 | 13 | 3 | 7 | 1 | 8 | 6 |
| 35 | 0 | 9 | 2 | 8 | 13 | 7 | 1 | 10 | 11 | 5 | 4 | 6 | 14 | 3 | 12 |

# Appendix C : Pairwise Comparisons of Logarithmized Inhibition Percentage Between Levels Within Each Parameter

**Table 22. Pairwise comparisons of logarithmized inhibition percentage between levels within the parameter of bandwidth**

| Pairwise Comparisons | | | | | | |
|---|---|---|---|---|---|---|
| (I) level | (J) level | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
| | | | | | Lower Bound | Upper Bound |
| Self-control condition | 1 bandwidth DL increased level | .015 | .008 | .511 | -.007 | .037 |
| | 1.5 bandwidth DLs increased level | .038* | .010 | .001 | .011 | .065 |
| | 2 bandwidth DLs increased level | 027* | .007 | .003 | .006 | .048 |
| | 3 bandwidth DLs increased level | .021 | .008 | .070 | -.001 | .044 |
| 1 bandwidth DL increased level | Self-control condition | .015 | .008 | .511 | -.037 | .007 |
| | 1.5 bandwidth DLs increased level | .023 | .010 | .198 | -.005 | .050 |
| | 2 bandwidth DLs increased level | .012 | .005 | .265 | -.003 | .027 |
| | 3 bandwidth DLs increased level | .006 | .008 | 1.000 | -.017 | .030 |
| 1.5 bandwidth DLs increased level | Self-control condition | .038* | .010 | .001 | -.065 | -.011 |
| | 1 bandwidth DL increased level | .023 | .010 | .198 | -.050 | .005 |
| | 2 bandwidth DLs increased level | .011 | .007 | 1.000 | -.031 | .010 |
| | 3 bandwidth DLs increased level | -.016 | .011 | 1.000 | -.048 | .015 |
| 2 bandwidth DLs increased level | Self-control condition | -.027* | .007 | .003 | -.048 | -.006 |
| | 1 bandwidth DL increased level | -.012 | .005 | .265 | -.027 | .003 |
| | 1.5 bandwidth DLs increased level | .011 | .007 | 1.000 | -.010 | .031 |
| | 3 bandwidth DLs increased level | .005 | .010 | 1.000 | -.033 | .023 |
| 3 bandwidth DLs increased level | Self-control condition | .021 | .008 | .070 | -.044 | .001 |
| | 1 bandwidth DL increased level | .006 | .008 | 1.000 | -.030 | .017 |
| | 1.5 bandwidth DLs increased level | .016 | .011 | 1.000 | -.015 | .048 |
| | 2 bandwidth DLs increased level | .005 | .010 | 1.000 | -.023 | .033 |

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

**Table 23. Pairwise comparisons of logarithmized inhibition percentage between levels within the parameter of frequency**

| Pairwise Comparisons | | | | | | |
|---|---|---|---|---|---|---|
| (I) level | (J) level | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
| | | | | | Lower Bound | Upper Bound |
| Self-control condition | 1 frequency DL increased level | .007 | .008 | 1.000 | -.016 | .030 |
| | 2 frequency DLs increased level | .042* | .008 | .000 | .020 | .064 |
| | 3 frequency DLs increased level | .004 | .009 | 1.000 | -.021 | .030 |
| | 4 frequency DLs increased level | .005 | .010 | 1.000 | -.023 | .032 |
| 1 frequency DL increased level | Self-control condition | -.007 | .008 | 1.000 | -.030 | .016 |
| | 2 frequency DLs increased level | .035* | .009 | .001 | .009 | .060 |
| | 3 frequency DLs increased level | -.003 | .006 | 1.000 | -.021 | .015 |
| | 4 frequency DLs increased level | -.003 | .011 | 1.000 | -.034 | .028 |
| 2 frequency DLs increased level | Self-control condition | -.042* | .008 | .000 | -.064 | -.020 |
| | 1 frequency DL increased level | -.035* | .009 | .001 | -.060 | -.009 |
| | 3 frequency DLs increased level | -.038* | .008 | .000 | -.062 | -.014 |
| | 4 frequency DLs increased level | -.038* | .013 | .042 | -.074 | -.001 |
| 3 frequency DLs increased level | Self-control condition | -.004 | .009 | 1.000 | -.030 | .021 |
| | 1 frequency DL increased level | .003 | .006 | 1.000 | -.015 | .021 |
| | 2 frequency DLs increased level | .038* | .008 | .000 | .014 | .062 |
| | 4 frequency DLs increased level | .000 | .011 | 1.000 | -.032 | .033 |
| 4 frequency DLs increased level | Self-control condition | -.005 | .010 | 1.000 | -.032 | .023 |
| | 1 frequency DL increased level | .003 | .011 | 1.000 | -.028 | .034 |
| | 2 frequency DLs increased level | .038* | .013 | .042 | .001 | .074 |
| | 3 frequency DLs increased level | .000 | .011 | 1.000 | -.033 | .032 |
| Based on estimated marginal means | | | | | | |
| *. The mean difference is significant at the .05 level. | | | | | | |
| b. Adjustment for multiple comparisons: Bonferroni. | | | | | | |

**Table 24. Pairwise comparisons of logarithmized inhibition percentage between levels within the parameter of intensity**

| Pairwise Comparisons | | | | | | |
|---|---|---|---|---|---|---|
| (I) level | (J) level | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
| | | | | | Lower Bound | Upper Bound |
| Self-control condition | 1 intensity DL increased level | .006 | .009 | 1.000 | -.020 | .033 |
| | 2 intensity DLs increased level | .020 | .008 | .166 | -.004 | .044 |
| | 3 intensity DLs increased level | .048* | .006 | .000 | .031 | .065 |
| | 4 intensity DLs increased level | .030* | .006 | .000 | .012 | .047 |
| 1 intensity DL increased level | Self-control condition | -.006 | .009 | 1.000 | -.033 | .020 |
| | 2 intensity DLs increased level | .014 | .009 | 1.000 | -.012 | .041 |
| | 3 intensity DLs increased level | .042* | .011 | .001 | .011 | .073 |
| | 4 intensity DLs increased level | .023 | .010 | .265 | -.006 | .053 |
| 2 intensity DLs increased level | Self-control condition | -.020 | .008 | .166 | -.044 | .004 |
| | 1 intensity DL increased level | -.014 | .009 | 1.000 | -.041 | .012 |
| | 3 intensity DLs increased level | .028* | .008 | .010 | .004 | .051 |
| | 4 intensity DLs increased level | .009 | .008 | 1.000 | -.013 | .032 |
| 3 intensity DLs increased level | Self-control condition | -.048* | .006 | .000 | -.065 | -.031 |
| | 1 intensity DL increased level | -.042* | .011 | .001 | -.073 | -.011 |
| | 2 intensity DLs increased level | -.028* | .008 | .010 | -.051 | -.004 |
| | 4 intensity DLs increased level | -.019 | .007 | .139 | -.040 | .003 |
| 4 intensity DLs increased level | Self-control condition | -.030* | .006 | .000 | -.047 | -.012 |
| | 1 intensity DL increased level | -.023 | .010 | .265 | -.053 | .006 |
| | 2 intensity DLs increased level | -.009 | .008 | 1.000 | -.032 | .013 |
| | 3 intensity DLs increased level | .019 | .007 | .139 | -.003 | .040 |

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

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