UNIVERSITY OF CALIFORNIA,
IRVINE

Psychophysical Study of Central Auditory Processing with Peripheral and Neural Deficits

DISSERTATION

submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in Biomedical Sciences

by

Carol Q. Pham

Dissertation Committee:
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2016

DEDICATION

For my parents.

For my loved ones.

And for XX and XY scientists abound.

❖❖❖❖❖

"It is imperfection - not perfection - that is the end result of the program written into that formidably complex engine that is the human brain, and of the influences exerted upon us by the environment and whoever takes care of us during the long years of our physical, psychological and intellectual development." – Rita Levi-Montalcini, *In Praise of Imperfection: My Life and Work*

"I was taught that the way of progress was neither swift nor easy." – Marie Curie

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ACKNOWLEDGMENTS

I feel incredibly fortunate to have the liberty of pursuing my scientific ambitions. My mission to understand the universe between our ears and brain would not be possible without the support and inspiration of others.

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My committee members, Professor John Middlebrooks, Professor Raju Metherate, Professor David Lyon, and Professor Leonard Kitzes provided invaluable support and academic advice throughout my graduate career. I learned from their guidance in scientific collaborations and mentorship. As neurophysiologists, they each inspire me to uphold the highest standards in science.

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and auditory brainstem responses in some of the auditory neuropathy subjects to help confirm or monitor their disorders prior to the gap detection experiments. Dr. Jonathan Venezia provided statistical support. Professor Arnold Starr provided valuable discussions about the data and I also thank him for his encouragement of my studies.

Past and present colleagues and collaborators at the Hearing and Speech (HESP) Laboratory and Center of Hearing Research contributed to the unique hearing science hub that shaped my scientific training. Professors Virginia Richards and Karina Cramer have been great role models as female scientists and gracious to review my research proposals throughout the years. Professor Yi Shen (former post-doc) and Dr. Thomas Lu provided valuable feedback on my advancement proposal. I am indebted to Tom Lu's technical assistance. A big thanks to Sahara, lab manager/yoga master, for providing research and administrative assistance and teaching me how to hang upside-down to relieve stress. I appreciate the camaderie of HESP labmates: Dr. Janice Chang, Dr. Dwight Wynne, Dr. Hsin-I Yang, Dr. Payton Lin, Jamal Alsmari, Matthew Richardson, and Katie Turner. I am grateful for all the ears and brains I borrowed for pilot experiments. A special thank you goes to all the research participants and their families for their dedication to our studies at large.

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Lastly, I am grateful for my loving parents and supportive family and friends who enrich my life. Mẹ, you empower me to be a strong woman like Dr. Carol after whom I am named. Bố, I share your joy of reveling in the cosmos and violin-playing. Thank you for reviewing my proposals and presentations. You encourage me see the silver lining in every cloud and inspire me to keep reaching for the stars, everyday.

To Tom, you inspire my best self. Thank you for the memories of our adventures through space and time. May the good times continue to roll!

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Pham CQ, Bremen P, Shen W, Yang S-M, Middlebrooks JC, Zeng F-G, Mc Laughlin M (2015) Central Auditory Processing of Temporal and Spectral-Variance Cues in Cochlear Implant Listeners. PLoS ONE 10:1-21.

Pham CQ, Zeng FG. Disrupted auditory nerve activity limits peripheral but not central temporal acuity (in preparation)

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Richardson M, Bremen P, **Pham CQ**, Middlebrooks JC, Zeng FG, McLaughlin M. Late Auditory Evoked Potentials in Responses to Informational Masking Stimuli. (in preparation)

Reviews

Zeng FG, **Pham CQ**: F1000Prime Recommendation of [Kral A, Hubka P, Held S, Tillein J. Brain 2013, 136(Pt 1):180-193; "Single-sided deafness leads to unilateral aural preference within an early sensitive period."]. In F1000Prime, 21 May 2013; DOI:10.3410/f.718008468.793476226.

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Rodgers CX, Vu V, Kochik SE, Zhang J, Shah A, Rustagi A, **Pham CQ**, Murphy J, Li T, Hsu T, Choi C, DeWeese MR. (2009). Developing a rodent model of selective auditory attention. In: Tucker-Davis Symposium on Advances and Perspectives in Auditory Neurophysiology; 2009 Oct 16; Chicago, IL.

TEACHING EXPERIENCE

2015 University of California Irvine, Department of Neurobiology & Behavior
Aspects of Mood Disorders
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ABSTRACT OF THE DISSERTATION

Psychophysical Study of Central Auditory Processing with Peripheral and Neural Deficits

By

Carol Q. Pham

Doctor of Philosophy in Biomedical Sciences

University of California at Irvine, 2016

Professor Fan-Gang Zeng, Chair

The present dissertation includes three studies collectively investigating perceptual consequences of peripheral and neural deficits in central auditory processes occurring in the ascending pathway from cochlea to cortex. Chapter 1 describes a study of how electrical stimulation delivered from a cochlear implant affects processing of co-varying frequency (spectral-variance) and temporal cues. We hypothesized post-lingually deaf cochlear implant listeners retain central processing abilities, which are hampered by degraded peripheral, cochlear inputs. In eight cochlear implant listeners, we measured auditory nerve compound action potentials to estimate peripheral filters, quantifying implant-induced spread of current and resultant spread of neural excitation. Then, we measured psychophysical detection thresholds in the presence of multi-electrode maskers placed either inside or outside the peripheral filter to determine peripheral and central contributions on processing. Results from actual and simulated implant listening support the hypothesis broad peripheral filters greatly limit central processing of spectral-variance, but not of temporal cues. Chapter 2 reports four experiments investigating effects of

nicotine (6 mg) gum on auditory tasks with varying attentional demand in healthy, normal hearing subjects. Lack of drug effects on central gain (tone-in-noise detection), temporal acuity (auditory gap detection), frequency resolution (spectral ripple discrimination), or auditory discrimination (attended listening) seem largely due to ceiling performance. Variability in the most demanding gap condition just reaching a significant decrease with nicotine could reflect improved temporal summation. Presumably, low task demand and maximum individual baseline attentional processing limit nicotine effects. Correlation analyses propose separability of attention and auditory processes and dissociable gap detection mechanisms. Chapter 3 describes a study in auditory neuropathy— disorders typically disrupting synaptic encoding and/or neural transmission of auditory signals in the cochlea and auditory nerve– differentiating temporal gap processes. The data seemingly support our hypothesis desynchronized neural discharges and/or reduced neural input limit peripheral temporal acuity but not central temporal acuity. Comparing subjects with disrupted auditory nerve activity with control subjects, a significant gap delay on the order of tens of milliseconds and insignificant delay on the order of hundreds of milliseconds may differentiate peripherally- and centrally-based temporal processing, respectively. We conclude by summarizing results and exploring future research for medical interventions.

CHAPTER 1

Central Auditory Processing of Temporal and Spectral-Variance Cues in Cochlear Implant Listeners

Part of this chapter has been published as:
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ABSTRACT

Cochlear implant (CI) listeners have difficulty understanding speech in complex listening environments. This deficit is thought to be largely due to peripheral encoding problems arising from current spread, which results in wide peripheral filters. In normal hearing (NH) listeners, central processing contributes to segregation of speech from competing sounds. We tested the hypothesis that basic central processing abilities are retained in post-lingually deaf CI listeners, but processing is hampered by degraded input from the periphery. In eight CI listeners, we measured auditory nerve compound action potentials to characterize peripheral filters. Then, we measured psychophysical detection thresholds in the presence of multi-electrode maskers placed either inside (peripheral masking) or outside (central masking) the peripheral filter. This was intended to distinguish peripheral from central contributions to signal detection. Introduction of temporal asynchrony between the signal and masker improved signal detection in both peripheral and central masking conditions for all CI listeners. Randomly varying components of the masker created spectral-variance cues, which seemed to benefit only two out of eight CI listeners. Contrastingly, the spectral-variance cues improved signal detection in all five NH listeners who listened to our CI simula- tion. Together these results indicate that widened peripheral filters significantly hamper central processing of spectral-

variance cues but not of temporal cues in post-lingually deaf CI listeners. As indicated by two CI listeners in our study, however, post-lingually deaf CI listeners may retain some central processing abilities similar to NH listeners.

GENERAL INTRODUCTION

The cochlear implant (CI) has become a gold standard for neural prosthetics as it is the only biomedical device capable of restoring a primary sense – hearing. In deaf individuals with loss of inner hair cell function, the implant converts acoustic sounds into electric signals. More specifically, the implant sends a train of electric pulses to electrodes threaded into the cochlea. These electrodes can stimulate spiral ganglion cells comprising the auditory nerve, bypassing the site of damage in the inner ear. Electrical stimulation of the auditory nerve conveys signals propagated through the ascending auditory pathway, which remarkably enables partially restored speech perception. A CI can have up to 22 intracochlear electrodes arranged tonotopically, each coding for a different range of frequencies (Zeng et al., 2008).

Cochlear implant (CI) listeners struggle to understand speech in complex environments whereas normal hearing (NH) listeners perform this task with apparent ease. In the latter group, sharp acoustic peripheral filters (Joris et al., 2011; Moore, 1985; Shera, et al., 2002; Unoki et al., 2006) allow for the resolution of individual harmonics (Bernstein & Oxenham, 2006) and permit separation of speech and interfering sounds into independent frequency channels (Dorman et al., 1998). The central auditory system can process strong spectral and temporal cues to group information from relevant frequency

channels and form auditory objects in auditory scene analysis (Bizley & Cohen, 2013; Bregman, 1990).

Compared to NH listeners, peripheral filters in CI users are much broader (Nelson, et al., 2008; Nelson et al., 2011) because current spreads out along the cochlea and excites a large population of auditory nerve fibers (Hartmann et al., 1984; Kral et al., 1998). This results in poor spectral resolution in the periphery and contributes to a CI user's inability to separate speech from interfering sounds (Fu & Nogaki, 2005; Stickney et al., 2004; Won et al., 2007). Electrical stimulation of the auditory nerve differs from acoustic stimulation in other notable ways. For example, acoustic stimulation generates stochastic firing patterns with phase-locking in the low frequency regions of the auditory nerve, whereas CI electrical-stimulation strategies cause entrainment in the nerve (i.e. action potentials strictly synchronized to the electrical pulses) up to rates of 800 Hz for stimuli 1-2 dB above threshold (Rainer et al., 1990; Javel, 1990; van den Honert & Stypulkowski, 1984, 1987). In addition to these differences in peripheral encoding, it is known that electrical stimulation causes neuroplastic changes in the central auditory system (Kral & Sharma, 2012). In spite of these central changes, we hypothesized that post-lingually deaf CI listeners may retain central processing abilities similar to NH listeners, but these abilities would be severely impaired by degraded peripheral encoding.

To test this hypothesis, we used multiple burst stimuli employed in standard informational masking paradigms (Kidd et al., 1994; Kidd et al., 2008) and customized them for CI listeners in a signal detection task. We used electrically evoked compound action potentials (ECAPs) to obtain a measure of peripheral filter bandwidth and then designed stimuli that elicited either predominantly peripheral or central (informational)

masking. We evaluated central processing abilities by calculating the difference in detection thresholds, i.e. central masking release, between maskers with and without spectral variance (randomly varying masker components) and/or temporal asynchrony (onset delays) which are cues thought to be accessible to the central auditory system of NH listeners (Kidd et al., 1994).

We conducted two experiments using these stimuli. Experiment I showed that CI listeners could use the temporal cues to gain release from central masking, indicating that central processing of temporal cues by CI listeners was similar to that in NH listeners. Unlike NH listeners, however, most CI listeners could not exploit the spectral-variance cues to gain central masking release. In Experiment II we simulated implant listening in NH listeners and showed that wide peripheral filters degraded the spectral-variance cues leaving the temporal cues intact. Furthermore, large inter-listener variability amongst both NH and CI listeners suggested intrinsic differences in central processing capabilities, which may affect sound segregation with degraded peripheral input.

In summary, all results indicated that central processing of the temporal cues in post-lingually deaf CI listeners is similar to that in NH listeners and is largely unaffected by peripheral encoding differences. Degraded peripheral encoding in CI listeners, however, likely limits the use of the spectral-variance cues by the central auditory system under electric stimulation.

GENERAL METHODS

Ethical statement

The University of California Irvine's Institutional Review Board approved all experimental procedures for both CI and NH listeners. Written informed consent was obtained from each listener and listeners were compensated for their participation on an hourly basis.

General experimental setup and procedure

All testing with CI and NH listeners was performed in a double-walled sound-attenuating booth.

The initial step was to measure threshold and comfort levels in all CI listeners to assess their dynamic ranges. To map comfort level, CI listeners judged signal loudness based on a standard 10-interval loudness scale (0 = no sound, 1 = barely audible, 6 = most comfortable, 10 = extremely loud). We asked CI listeners to indicate when they perceived the given stimulus to be most comfortable (6/10). Comfort level represented a conservative measure of loudness to avoid overstimulation with multi-component stimuli. We used a standard 3-down-1-up, two-alternative force choice (2AFC) paradigm (Levitt, 1971) to track thresholds of unmasked signals (threshold level of CI listeners) and calculated the dynamic range as the difference between comfort and threshold level per intracochlear electrode.

We used the same 2AFC procedure to measure psychophysical detection thresholds of masked signals in Experiments I (CI listeners) and II (NH listeners). For CI listeners, we set the starting level to the listener's comfort level. To accelerate threshold convergence,

we set the signal level in consecutive repetitions to 40% percent of the dynamic range of the previously tracked threshold with the provision that the starting level could never exceed the comfort level. The subject listened to two intervals indicated by buttons labeled '1' and '2' on a graphical user interface (MATLAB, The Mathworks, Natick, MA). The buttons sequentially illuminated as the stimuli played. By random selection, one interval contained the signal and masker while the other interval contained the masker alone. We instructed listeners to select the interval that contained the signal via mouse click. As visual feedback after each trial, the interval button turned green or red to indicate a correct or an incorrect response, respectively. We calculated signal detection threshold as the average over the last six out of ten reversals.

Statistics and data analysis

Absolute signal detection thresholds across listeners were not normally distributed. Therefore, a non-parametric, repeated measures, Friedman's test was used to compare masker conditions. A statistical significance level of $p < 0.05$ was used with *post-hoc* Bonferroni adjustments. We used linear regression with a least-squares criterion to assess correlations between masker conditions and a two-sample Kolmogorov-Smirnov test to determine significant differences ($p < 0.05$) between paired conditions. All statistical analysis was performed using MATLAB.

EXPERIMENT I: SIGNAL DETECTION WITH PERIPHERAL AND CENTRAL MASKERS

RATIONALE

A number of studies in NH listeners have used non-speech, multi-tone maskers with a protected band centered at the signal frequency (Bremen & Middlebrooks, 2013; Kidd et al., 1994; Neff et al., 1993; Neff & Callaghan, 1988; Neff & Green, 1987) to study the effects of energetic and informational masking. Energetic masking is defined as masking due to the excitation of overlapping neural populations. Although the term informational masking remains elusive, it is traditionally defined negatively as any masking that is non-energetic in origin (Durlach et al., 2003). In CI listeners, peripheral filters (i.e. a measure of cochlear spread of neural excitation) can be measured using ECAPs (Brown et al., 1990; Lai & Dillier, 2000; Abbas et al., 1999). In the present study, we used this filter to define the terms 'peripheral' and 'central' masking which are parallel to, but not strictly synonymous with, the terms 'energetic' and 'informational' masking. Consequently, we define peripheral masking as an increase in detection threshold of a signal electrode due to the presence of masking electrodes located within the peripheral filter of the signal. Furthermore, we define central masking as an increase in detection threshold of a signal electrode due to stimulation of masking electrodes located outside the same peripheral filter.

Results from previous studies (Bremen & Middlebrooks, 2013; Kidd et al., 1994; Neff et al., 1993; Neff & Callaghan, 1988; Neff & Green, 1987) indicated that NH listeners can experience large amounts of central masking and that manipulation of the temporal and spectral content of the stimuli can facilitate central masking release (i.e., decrease signal detection thresholds using stimulus cues). Experiment I had three goals: 1) to determine if CI listeners experience similar amounts of central masking as NH listeners; 2)

to determine if CI listeners could access similar temporal and spectral-variance cues to gain release from central masking; and 3) to identify peripheral encoding mechanisms which may impair central processing.

EXPERIMENT I. METHODS

Cochlear Implant listeners

We screened 22 implants in fifteen CI listeners, including 7 bilateral implant listeners, with Nucleus 24, Nucleus 5, or Freedom devices (Cochlear Corporation, Australia) to meet three inclusion criteria. First, CI listeners needed to have a dynamic range greater than 20 clinical units to enable testing of different masker levels. Second, CI listeners needed to have a measurable ECAP filter on electrode 11 (signal electrode) that could be well fit by a Gaussian function ($R^2 \geq 0.80$) with a standard deviation $\leq \pm 7$ electrodes so that multi-electrode maskers could be placed either inside or outside the peripheral filter. Third, our sample of CI listeners had to have 22 active electrodes switched on to enable testing of different masker electrode configurations. Out of the fifteen listeners screened, two listeners failed the first criterion, three listeners failed the second criterion (two listeners had filter widths $>\sim \pm 8$ on electrode 11; one listener did not have measurable ECAPs) and two listeners failed the third criterion. Eight post-lingually deaf, adult listeners met all three criteria (five females; age 51-85; mean age 70). Table 1.1 provides additional demographic and audiological details for these eight listeners.

Listener	Sex	Age	HL age	Deafness duration (y)	Etiology	HA (y)	CI (y)	CI device (no. of CI)	DR signal E11 (uA)
CI 1	F	70	45	25	prebycusis	53	3	Freedom (1)	435
CI 2	F	75	30	25	hereditary	33	8	N24 (2)	183
CI 3	M	78	63	7	acoustic trauma	45	6	N24 (2)	148
CI 4	F	74	35	16	hereditary	35	2	N24 (2)	187
CI 5	M	85	58	23	hereditary	60	3	N24 (2)	210
CI 6	F	51	18	32	hereditary	20	2	Freedom (2)	296
CI 7	M	53	15	38	otosclerosis autoimmune disease	23	2	N5 (2)	185
CI 8	F	70	15	50	otoxicity	39	8	Freedom (2)	225

Table 1.1. CI listeners' demographic and audiological details
Age, refers to age during experimental testing; HL age, refers to age of hearing loss onset; deafness duration, refers to number of years since the onset of profound hearing loss; HA, refers to number of years of hearing aid usage in the tested ear; CI, refers to number of years of cochlear implant usage in the tested ear; CI device (number of CI), Freedom means a Nucleus Freedom device, N24 and N5 means a Nucleus 24 and Nucleus 5 device, respectively; DR signal E11 (μA), dynamic range of stimulus signal presented on electrode 11 in microamperes.

Peripheral filter measurement using Electrically Evoked Compound Action Potentials

We used Custom Sound EP (Cochlear Corporation, Australia) software to record ECAPs in the CI listeners. We employed the forward masking protocol previously described by Brown and colleagues (Brown et al., 1990), which uses a subtraction technique in a masker-probe paradigm to separate the ECAP from the stimulation artifact. To capture the spread of excitation along the cochlea, we moved the masker across the electrode array while we fixed the probe and recording electrodes. Extracting the N1-P1 amplitude of separate ECAP responses measured at each masker electrode location gave a measurement of one peripheral filter. The protocol used charge-balanced, biphasic pulses delivered in monopolar stimulation mode through the listener's implant. Recording parameters were optimized for eliciting neural responses per CI listener. We used typically a pulse duration of 25 μs/phase, an interphase gap of 7 μs and a pulse rate of 40 (probe) and 100 (masker) pulses/s and set the delay between masker and probe pulse to 400 μs. Both masker and probe pulse amplitude were set to the listener's most comfortable loudness level. We used

the extracochlear electrode MP1 as the reference electrode and an intracochlear electrode as the recording electrode, the latter offset by two positions from the probe electrode. The delay between probe pulse and recording buffer was on average 100 μs and we set the amplifier gain to either 40 or 50 dB. The Custom Sound EP software automatically extracted N1-P1 ECAP amplitudes (Lai & Dillier, 2000), which we inspected visually and corrected manually when necessary. A characterization of CI peripheral filters is shown in Fig 1.1 including examples of filter fits for three probes tested in one CI listener CI 5.

Quantifying peripheral ECAP filters

To quantify peripheral filters, we exported ECAP N1-P1 amplitudes from Custom Sound EP and fitted them with a Gaussian curve using the lsqcurvefit function in MATLAB. The following formula describes the Gaussian fit,

$$y = ae^{-(x-\mu)/\sigma 2} \quad \text{(Eq. 1)}$$

where a is the amplitude of the filter, x is the masker electrode number, μ is the mean (i.e. probe electrode), and σ is the standard deviation (i.e. bandwidth of the ECAP filter). To facilitate comparison between filters of an individual listener, we normalized them by dividing by the maximum amplitude of a given filter fit (Fig 1.1).

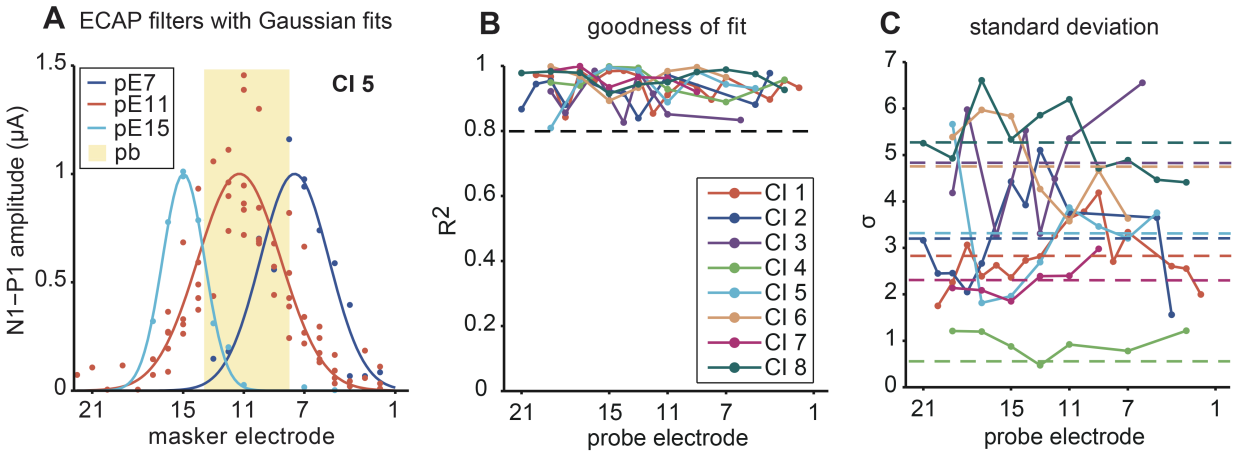

Figure 1.1. Characterization of CI peripheral filters. A Electrically evoked compound action potential (ECAP) filters with Gaussian fits. Spread of excitation profiles are shown for three probe electrodes (pE7, pE11, pE15) in listener CI 5. ECAP N1-P1 amplitudes and Gaussian fits are indicated with circles and lines, respectively. **B** Goodness of fit. R^2 between the measured filter and the Gaussian function for each tested probe electrode in all CI listeners (different colors) are shown. The black dashed line demarcates where fits are equivalent or higher than R^2= 0.8. **C** Standard deviation. Peripheral filter bandwidth (σ) was estimated as a function of probe electrode. Each colored, dashed line corresponds to the average standard deviation across probes per CI listener. Based on these data we estimated a protected band (pb; shaded region in A) of ±3 electrodes to best separate peripheral and central masking effects (see text for details).

Electrical stimulation and electrode mapping for perceptual studies

We presented all electrical stimuli using a research interface (HEINRI) (Wygonski & Robert, 2002). In four bilateral CI listeners (CI 4,6,7,8) we stimulated the second implanted ear and in the two other bilateral cases (CI 2,3) we stimulated the first implanted ear after measuring ECAPs in both ears. We tested the second implanted ear for the following reasons: the electrode array was not fully inserted in the first implanted ear (CI 4); the first implant had inactive electrodes (CI 6, CI 8); the second CI had sharper ECAP filters (CI 7). We set the extracochlear electrodes, MP1 and MP2, as return electrodes and stimulated in monopolar mode. The pulse width, interphase gap and pulse rate remained fixed for all stimuli at 25 μs/phase with a 10-μs interphase gap and a pulse rate of 300 pps per channel. When multiple electrodes were stimulated we used continuous interleaved sampling with

the pulse on the most basal electrode occurring first, and the following pulse on the next electrode in the apical direction, occurring 125 μs after the onset of the previous pulse.

Using a signal matched to that used with the peripheral and central masking stimuli (see below), we mapped comfort and threshold levels on all electrodes. The signal consisted of four bursts of a 40-ms pulse train presented on one electrode at a rate of 5/s. In the first step, we decreased stimulation level by 5 clinical units, 3 clinical units in the two subsequent steps and 1 clinical unit increments thereafter. We determined the threshold level as the average of the last six out of ten reversals. A map of dynamic range was created and used for subsequent testing. We checked these maps periodically, but did not observe significant shifts during the testing period.

The HEINRI system allows specification of pulse amplitude levels in clinical units (CU; range 0 to 255 in steps of 1) which is related to current (I) by the following formula,

$$I = 10\,\mu A * 175^{CU/255} \qquad \text{(Eq. 2)}$$

During all threshold tracking runs, current levels were controlled, adjusted and stored in CU. In the results section, we report tracked thresholds in units of current (μA) derived from Eq 2.

Peripheral and central masking stimuli

We designed stimuli that intended to separate peripheral and central masking effects. Based on the ECAP peripheral filter measurements, we estimated filter bandwidths as the standard deviation (σ) of the Gaussian fit (shaded protected band (pb), Fig 1.1A). In all experiments, the signal was presented on electrode 11. Both the signal and masker were 40-ms bursts pulsed at a rate of 5/s. Maskers comprised of four electrode components per

burst. Masker components could either be within (peripheral masker, Fig 1.2A, D) or outside (central masker, Fig 1.2B-C, E-F) the ECAP filter.

To study central masking release based on spectral variance across masker bursts (i.e. spectral-variance release), we compared conditions in which the four masker electrode positions remained constant between bursts (C masker, Fig 1.2B, E) with conditions in which the four masker electrode positions varied randomly between bursts (R masker, Fig 1.2C, F) over time. In NH listeners the R-like stimulus yields lower signal detection thresholds when compared to C-like stimulus (Bremen & Middlebrooks, 2013; Kidd et al., 1994). Peripheral (P) masker components remained constant between bursts due to the restricted number of distinct electrodes within the protected band.

Additionally, we examined masking releasing due to a temporal cue (i.e. temporal release) by presenting maskers both synchronously and asynchronously *re* signal in separate conditions. Note that the delay between biphasic pulses on different electrodes was 125 μs, leading to a slight temporal offset among bursts on various electrodes even in the nominally synchronous condition, but this delay was negligible compared with delays on the order of milliseconds between bursts in the asynchronous condition. In the synchronous conditions, the onset of the four masker bursts and the signal was synchronous (given the technical limitations of the stimulation device) (Fig 1.2A-C). In the asynchronous conditions, each masker burst had a random onset delay of 0, 50, 100 and 150 ms *re* signal (Fig 1.2D-F). Thus, in asynchronous conditions, one masker component overlapped with the signal and the other three were separated in time from the signal and from each other. This yielded a total of six masking stimuli: peripheral (P) and central (C, R) maskers in synchronous (sync) and asynchronous (async) timing conditions (this

convention for labeling the stimulus condition will be used henceforth). We selected stimulus parameter settings to closely match those previously tested in NH listeners (Bremen & Middlebrooks, 2013) for the purpose of comparing masked signal detection in electric vs. acoustic hearing.

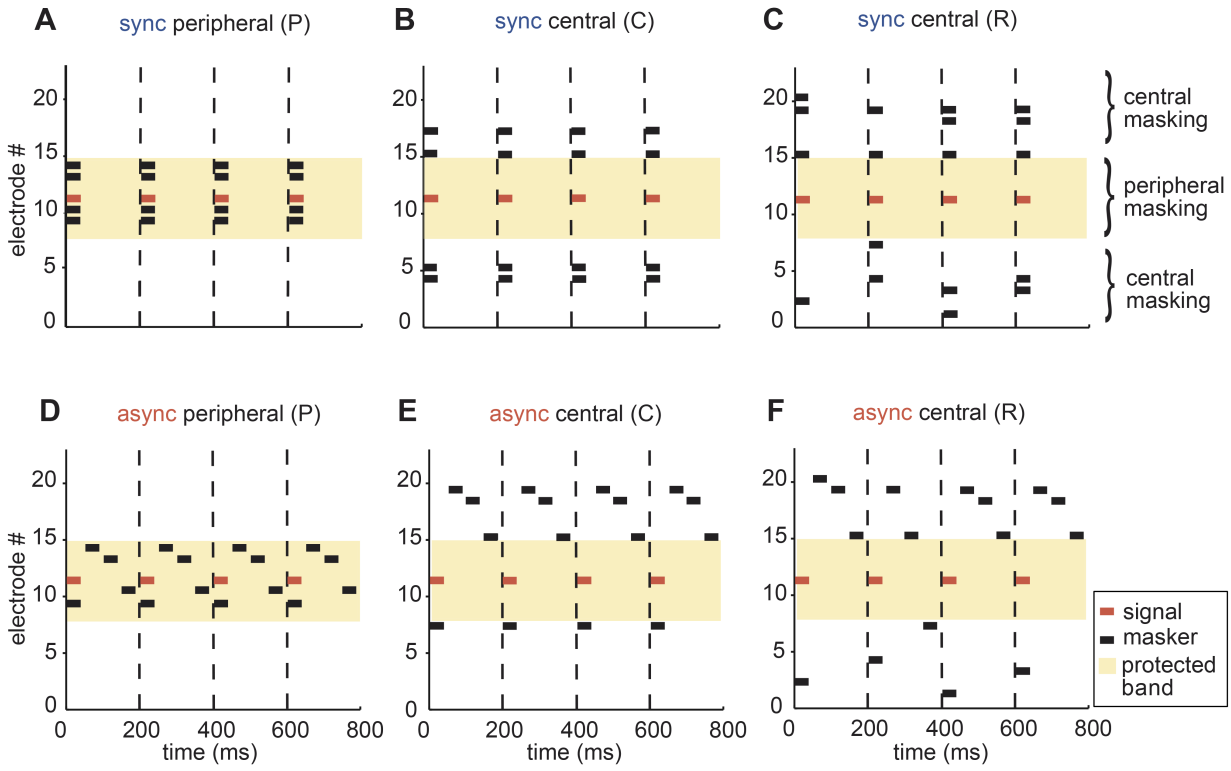

Figure 1.2. Peripheral and central masking stimuli tested in CI listeners. Multi-electrode maskers (black) were placed either inside (peripheral masking; P masker: **A**, **D**) or outside (central masking: **B**, **C**, **E**, **F**) the peripheral filter (marked by the protected band, shaded region). The signal (red) was fixed on electrode 11. Four randomly chosen masker electrode positions outside of the filter remained constant (C masker: **B**, **E**) or randomly varied (R masker: **C**, **F**) between bursts over time. Maskers were presented in two temporal conditions. In the synchronous condition (sync maskers: **A-C**) masker components gated on relatively synchronously re signal and in the asynchronous condition (async maskers: **D-F**) the four independent masker components had onset delays of 0, 50, 100, and 150 ms re signal (see text for details).

Using ECAP filters to separate peripheral and central masking effects

We used filters derived from ECAP measurements to determine the degree to which each electrode contributed to either peripheral or central masking. ECAPs were measured with the masker and probe electrode fixed at the most comfortable level on the test electrode. During threshold tracking experiments, the masker electrodes were fixed at 10%, 30% or 50% DR (dynamic range of each masking component) while the signal electrode started at comfort level and was generally tracked at a threshold below this level. Note that as the current level decreases so does the spread of neural excitation, meaning that the excitation profiles elicited during threshold tracking experiments will be narrower than those measured in the ECAP experiment. Thus, for central masking stimuli, it is reasonable to assume that masking electrodes outside the protected band excite a different auditory nerve fiber population than that responding to the signal electrode inside the protected band. For the peripheral masking stimuli, however, it is likely that electrodes placed inside the protected band stimulate a nerve fiber population that mostly, but not completely, overlaps with the population excited by the signal electrode. This means that the peripheral masking stimuli can potentially create some limited central masking effects. A limitation of relating ECAPs to behavioral masking may be that response amplitudes do not exclusively represent the spread of excitation. Although the amplitude of the ECAP can approximate the number of neurons responsive to a specific stimulus, this method assumes that the recording electrode primarily measures the response from neurons near that electrode without full determination of the degree of spatial filtering. Consequently, the response amplitude as a function of recording positioning for a fixed level and position of stimulating electrode depends on the spread of excitation across fibers and the spread of

the response fields from each active neuron to the recording electrode (Abbas & Miller, 2004).

2AFC paradigm for testing CI listeners

For all tests we held the masker level constant at a fixed percentage of the dynamic range and adjusted the signal level using the 2AFC threshold tracking procedure. We used a protected band of ±3, ±5, or ±7 electrodes centered at the signal electrode and measured detection thresholds at three different masker levels (10%, 30% and 50% DR; only 30% DR was tested with different protected bands).

Like in the initial mapping of threshold level, the signal level decreased by 5, 3, 1 clinical units for the first, second and third, and the subsequent reversals, respectively. We repeated threshold measurements five times per listener. Each interval within a trial contained maskers with freshly drawn electrode components. We presented all six stimuli randomly during a session (3-5 repetitions per masker condition lasting about 240-300 mins with at least 10-min breaks after every 30 mins of testing) and held the masker condition constant during one threshold measurement (~100 trials lasting about 5 mins). Each of the six maskers was tested before any condition was repeated. We roved the level of the masker-only interval by ±3 clinical units to minimize the contribution of level cues between the masker-only and the signal-plus-masker intervals (four vs. five electrodes).

Training

To minimize learning effects, all listeners underwent two types of training sessions prior to data collection. In the first training session, listeners used a graphical user

interface to listen to the signal alone, all six maskers alone, and each signal-plus-masker condition to become familiar with the stimuli. The second training session used the threshold tracking paradigm outlined above with the masker level set to 10% of the dynamic range. We trained listeners using the asynchronous R masker, generally the easiest masker condition, to help listeners become accustomed to the test procedure.

Quantifying spectral-variance release from central masking

We calculated the amount of spectral-variance release from central masking by subtracting median R from median C thresholds (Figs 1.5, 1.7, 1.9). In order to assess the amount of variability, we used a bootstrapping method. First, we randomly drew three repetitions out of the total five threshold measurements with replacement and calculated the mean over these repetitions. We repeated this procedure 1000 times and finally calculated the standard deviation over these bootstrapped means. We also applied this analysis to NH data from Experiment II. The error bars in Figs 1.5, 1.7 and 1.9 indicate the bootstrapped standard deviation.

EXPERIMENT I. RESULTS

ECAP spread of excitation

Fig 1A shows the ECAP filters in one listener (CI 5) with the N1-P1 amplitudes (circles) and corresponding Gaussian fits (lines). This figure shows filters measured with the probe electrode (pE) at three different locations (electrode 7, 11 and 15). Fig 1B summarizes the goodness of fit between the measured filters and the Gaussian function (Eq. 1) for each of the eight CI listeners who participated in the study. In all listeners, the

spread of excitation profiles were measured for a range of different probe electrodes spanning the length of the electrode array.

Gaussian fits for 80 out of 103 probe electrodes yielded $R^2 \geq 0.8$ (Fig 1.1B) whereas the other 23 probes were edge electrodes yielding weak or no neural responses. Initial visual inspection suggested that a Gaussian model would be a reasonable approximation of individual's filter shape and high R^2 values justified use of this simple model. For the purpose of estimating bandwidth for stimulus generation (criteria for designating electrode positions inside vs. outside the peripheral filter), the Gaussian model provided the best approximation of filter shape without resorting to complicated multi-parameter models.

Even after careful subject selection, fitted ECAP filter bandwidth (standard deviation; σ in Eq. 1) varied largely across listeners and electrode, ranging from 1-7 electrodes wide (Fig 1.1C, colored dashed lines). Listener CI 4 had the narrowest filters with standard deviations close to 1, whereas listener CI 8 tended to have the widest filters. The three different protected bandwidths selected for testing (±3, ±5 and ±7 electrodes) helped to accommodate for some of this variability. The influence of the protected bandwidth on central and peripheral masking is further explained below and is illustrated here with the following examples: At one extreme in listener CI 8, a protected band of ±3 electrodes may not have been wide enough to ensure that central masking stimuli did not create some unwanted peripheral masking. In this listener, a protected band of ±7 electrodes would have minimized peripheral masking effects with the central masking stimuli. At the other extreme in listener CI 4, a protected band of ±3 electrodes may have meant that the peripheral masking stimuli created some unwanted central masking effects.

Because the peripheral masking stimuli required a bandwidth of at least five electrodes (one signal plus four masker electrodes) a narrower protected band was not tested. Thus, the protected band may not have always completely distinguished central and peripheral masking effects in all listeners. It did, however, always limit any unwanted peripheral masking effects when central masking stimuli were presented and vice-versa.

Signal detection with peripheral and central maskers

Fig 1.3 presents the results from the six stimulus conditions at three masker levels (10%, 30% and 50% DR shown in A-C, respectively) using a protected band (pb) of ±3 electrodes. Each listener's median detection threshold for a specific condition, hereafter referred to as a detection threshold, is shown as a different symbol. Individual repetitions are shown as gray x's. The midline of the boxes indicates the group median and the lower and upper lines indicate the 25^{th} and 75^{th} quartiles, respectively (red and blue indicate asynchronous and synchronous conditions, respectively). Absolute comfort and threshold levels varied widely across CI listeners resulting in greatly varying absolute detection thresholds (not shown). For that reason, each threshold was referenced to the listener's detection threshold in the synchronous C condition per masker level (dashed lines, Fig 1.3). As a result, median synchronous C thresholds per CI listener were plotted as zero in each plot with negative detection thresholds indicating masking release (i.e. better performance) relative to this generally most difficult condition.

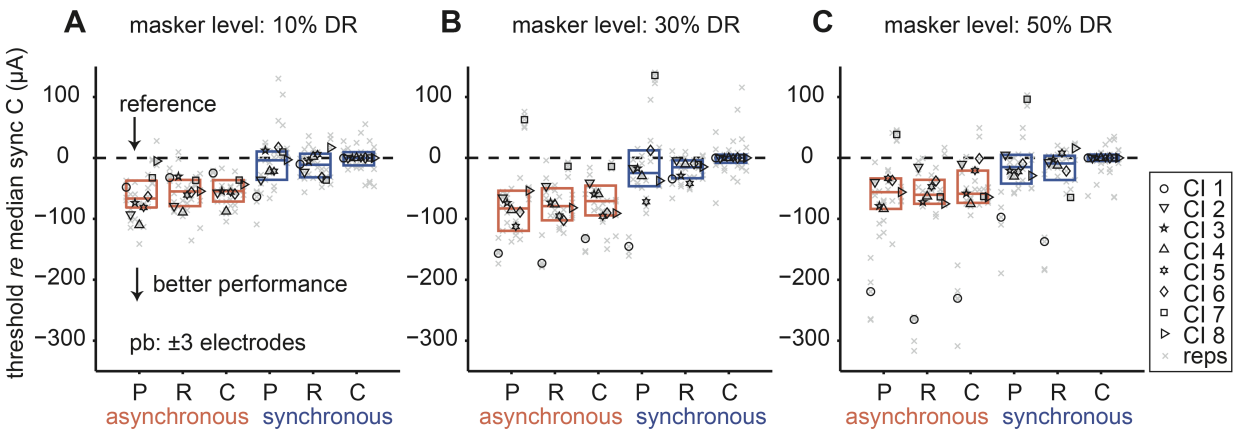

Figure 1.3. Effect of masker level on signal detection thresholds. Capital letters on abscissa correspond to peripheral (P) and central (C, R) maskers in both asynchronous (red) and synchronous (blue) timing conditions. Signal detection thresholds (re each CI listener's median, synchronous C threshold) are plotted as a function of stimulus. Detection thresholds are expressed in current (μA) and were tracked at three masker levels expressed as a percentage of dynamic range: **A** 10%, **B** 30% and **C** 50% DR. The protected band (pb) was set to ±3 electrodes. Different symbols indicate individual listeners' median thresholds and gray x's indicate individual repetitions. The dashed line marks the reference point. The mid-line of the boxes indicate the group median and the lower and upper lines indicate the 25th and 75th quartiles, respectively. Lower values indicate less masking (i.e. better performance).

Temporal release

Gating the maskers on asynchronously introduced a temporal cue in the stimuli that could potentially facilitate signal detection. It was clear across all masker levels that the asynchronous condition produced lower detection thresholds than the synchronous condition (Fig 1.3). A Friedman's test with correction for multiple comparisons showed that this effect was significant ($p < 0.01$). A correlation analysis further quantified this effect for thresholds in both timing conditions. We plotted individual absolute thresholds from synchronous conditions as a function of the corresponding asynchronous conditions with paired repetitions within the same masker level condition and timing condition (Fig 1.4A). Ninety-two percent of the points fell above the unity line, meaning that the asynchronous condition yielded significantly lower detection thresholds than the synchronous condition (Kolmogorov-Smirnov test, $p << 0.01$). All listeners experienced

greater masking with synchronous than asynchronous maskers (>90% of points above the unity line). A linear regression ($R^2 = 0.90$; $p < 10^{-5}$) yielded an offset of about 63 µA between synchronous over asynchronous timing conditions and further support that temporal cues facilitate signal detection. The effect was most pronounced in listener CI 1 (red). Furthermore, this analysis suggested that a temporal cue could facilitate release from both peripheral and central masking (Fig 1.3). The finding that temporal cues could facilitate signal detection in complex listening situations is in agreement with reports using similar stimuli in NH listeners (e.g. Bremen & Middlebrooks, 2013) as well as auditory scene analysis studies in CI listeners (Chatterjee et al., 2006; Hong & Turner, 2009).

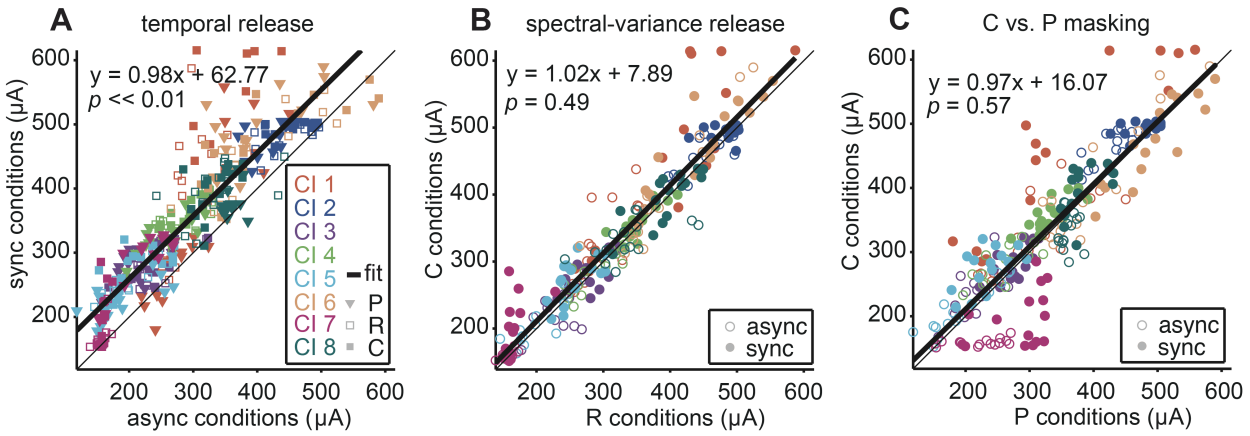

Figure 1.4. Correlation analysis quantifying temporal and spectral-variance release. In all plots, individual listeners are indicated by different colors. Data from all masker levels with a protected band of ±3 electrodes are plotted. Repetitions were paired within the same masker level and timing condition. The unity line and a linear fit through the data = are shown as thin and thick lines, respectively. Each plot shows the equation describing the linear fit and the p value from the Kolmogorov-Smirnov test. All linear fits were significant, $p > 10^5$, with $R^2 = 0.90$, 0.96, and 0.89 in **A**, **B**, and **C** respectively. **A** Temporal release. Absolute detection thresholds with synchronous (sync) maskers are shown as a function of thresholds with asynchronous (async) maskers. Inverted triangles represent peripheral (P) maskers whereas open and filled squares represent central (C, R) maskers, respectively. **B** Spectral-variance release. Absolute detection thresholds with C maskers are shown as a function of thresholds with R maskers. Open and filled circles represent async and sync timing conditions, respectively. **C** Central vs. Peripheral masking. Absolute detection thresholds with C maskers are shown as a function of thresholds with P maskers. Open and filled circles represent async and sync timing conditions, respectively.

Spectral-variance release

In NH listeners, spectral variance in addition to a temporal cue can facilitate central masking release (Bremen & Middlebrooks, 2013; Kidd et al.,, 1995; Kidd et al., 1994). Spectral variance in the present experiment was introduced by randomly varying electrode positions (frequencies) in the central masker across bursts (R masker) while the signal remained constant across bursts. Across masker level and timing conditions, there was a weak non-significant tendency for the R thresholds to be lower than the C thresholds (Fig 1.3) ($p > 0.05$; Friedman's test using the Bonferroni correction for multiple comparisons).

Fig 1.4B displays thresholds for all central (C and R) conditions in the same scatter plot format as used previously. Sixty-five percent of the points fell above the unity line, indicating that C and R detection thresholds were similar, but that there was a weak tendency for C thresholds to be higher than R thresholds with large individual differences ranging from 47%-83% of points above the unity line. A linear fit to the data showed a small offset of about 8 μA between the central masking conditions ($R^2 = 0.96$; $p < 10^{-5}$). In general, spectral variance did not facilitate central masking release in most CI listeners (Kolmogorov-Smirnov test, $p = 0.49$). Listeners CI 1 (circle), CI 7 (square), and to a lesser degree CI 6 (diamond) were notable exceptions in that their detection thresholds were similar to those seen in NH listeners with lower R thresholds relative to P and C thresholds at all masker levels (Fig 1.3). For listener CI 6, this pattern existed only at 10% masker level. These results failed to reach significance.

We further analyzed performance by calculating the difference between thresholds obtained with the C and R maskers as a measure of spectral-variance release. These differences were plotted as a function of masker level for the asynchronous (Fig 1.5A) and

synchronous (Fig 1.5B) conditions with higher values signifying more release. Most listeners, except for listeners CI 1 and CI 7, showed negligible amounts (close to 0 μA) of spectral-variance release independent of masker level and temporal condition. Note, however, that some listeners exhibited a peaked function (e.g. asynchronous: CI 4 and synchronous: CI 3 and CI 5) indicating modest amounts of spectral-variance release at the 30% masker level. In contrast, listener CI 1 exhibited a large increase in spectral-variance release across masker levels. In the asynchronous condition, spectral-variance release for listener CI 6 increased linearly with masker level and reached a maximum of ~40 μA. Spectral-variance release in listeners CI 1 and CI 3 increased between 10% to 30% DR masker level and plateaued at 50% DR masker level. In the synchronous condition, spectral-variance release for that listener reached ~160 μA. Note that the bootstrapped standard deviations could be quite large with increasing masker level (e.g. 30% DR: CI 1, 6, 7; 50% DR: CI 1, 7) (Fig 1.5B). This variability might be related to task difficulty (Choi et al., 2014). The difference in spectral-variance release between the two temporal conditions is most likely due to ceiling and floor effects. That is, in the asynchronous conditions the listener performed close to ceiling so that addition of spectral-variance only slightly decreased detection thresholds. In the synchronous conditions, however, the listener operated at floor performance so additional cues could strongly decrease the signal detection threshold (Bremen & Middlebrooks, 2013).

Central versus peripheral masking

There was no significant difference between C and P thresholds, irrespective of the temporal condition and masker level (Fig 1.3) ($p > 0.05$; Friedman's test with Bonferroni

correction for multiple comparisons). Fig 1.4C displays thresholds in the same scatter plot format as used previously, with individual absolute thresholds from C conditions plotted as a function of the corresponding P conditions. A linear fit to the data showed a small offset of about 16 μA ($R^2 = 0.89$; $p < 10^{-5}$). Sixty-two percent of the points fell above the unity line indicating a small tendency for C thresholds to be higher than P thresholds but this difference was not significant (Kolmogorov-Smirnov test, $p = 0.57$). In contrast, listeners CI 6 and CI 7 showed higher P than C thresholds (<40% of points above the unity line).

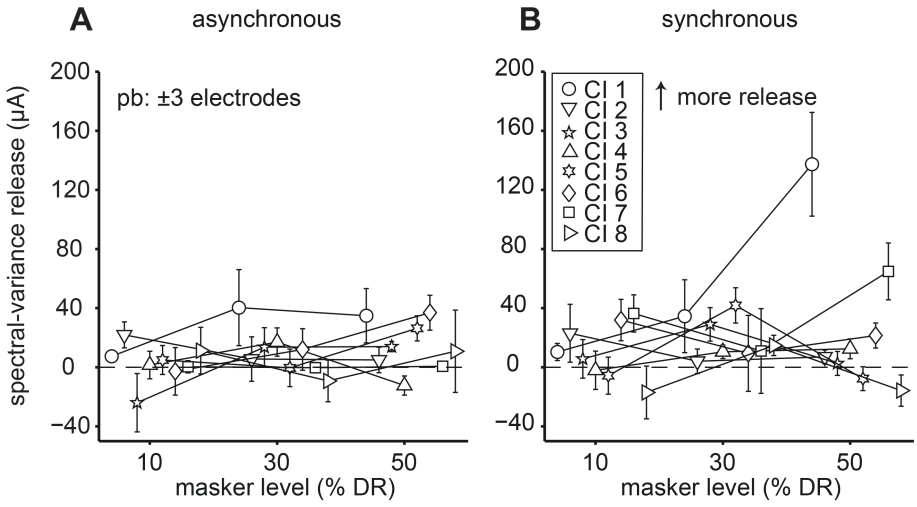

Figure 1.5. Effect of masker level on spectral-variance release. Data from all masker levels with a protected band (pb) of ±3 electrodes are plotted. **A** Asynchronous conditions. **B** Synchronous conditions. The amount of spectral-variance release equals the difference between median central (C-R) thresholds. The dashed line at zero demarcates no difference in central thresholds as a function of masker level (percentage of dynamic range, % DR). Different symbols represent individual listeners. Error bars indicate the standard deviation obtained with a bootstrap method (see main text). Higher values indicate more spectral-variance release.

Absolute loudness is not a reliable cue for masking release

We wondered if threshold differences between the three different masker types (P, C, and R) could be due to differences in loudness of these maskers. Accordingly, we performed a subjective 2-interval loudness comparison experiment with our CI listeners.

We tested pairs of maskers, e.g. P vs. C masker, etc., and asked the listeners to indicate which of the two intervals contained the louder sound. We did not find any systematic differences in their loudness judgments of the maskers. Therefore we excluded loudness differences as a major contributing factor to the observed detection thresholds (data not shown).

Increasing protected bandwidth fails to improve spectral-variance release

With a protected band of ±3 electrodes two CI listeners showed clear spectral-variance release from central masking (Fig 1.5B, CI 1, 7). We wondered whether increasing the protected band might enhance spectral-variance release. Fig 1.6 shows the effect of increased protected band from ±3 (re-plotted from Fig 1.3B) to ±5 and ±7 electrodes (Fig 1.6A-C, respectively) at the same fixed 30% masker level. In general, the threshold difference between the two timing conditions persisted irrespective of the protected bandwidth (Friedman test, $p < 0.05$ for all comparisons at the group level). Peripheral thresholds were not significantly different from either central C or R thresholds (Friedman test, $p < 0.05$ for all comparisons). At ±7 protected band, P thresholds tended to be higher than both central C and R thresholds. Interestingly, four listeners (CI 1, 6, 7, 8) had a higher P detection thresholds compared to C and R thresholds in both temporal conditions, respectively (Fig 1.6C). In listener CI 7, increasing the protected band to ±5 and ±7 significantly increased differences between asynchronous P and C thresholds (Friedman test, $p < 0.05$).

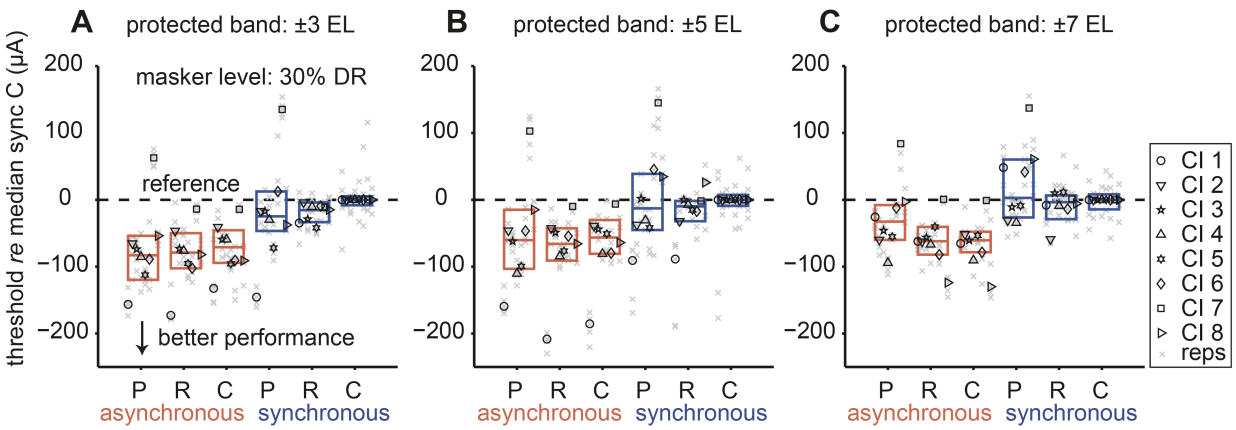

Figure 1.6. Effect of increased protected band on signal detection thresholds. Capital letters on abscissa correspond peripheral (P) and central (C, R) maskers in both timing conditions. Signal detection thresholds (re each CI listener's median, synchronous C threshold) are plotted as a function of stimulus. Three protected band conditions were tested at a masker level of 30% dynamic range (DR): **A** ±3, **B** ±5 and **C** ±7 electrodes (EL). All other conventions are as in Figure 3.

The amount of spectral-variance release from central masking as a function of protected bandwidth is shown in Fig 1.7. In the asynchronous conditions (Fig 1.7A) spectral-variance release either stayed constant (CI 2, 3, 7, 8), decreased slightly (CI 1, 4), peaked (CI 5) or decreased (CI 6) at ±5 protected bandwidth (CI 5). We saw similar trends in the synchronous conditions (Fig 7B) in that spectral-variance release could stay constant (CI 4, 6, 7), decrease (CI 3, 5), increase (CI 2), peak (CI 1) or decrease (CI 8) at a protected bandwidth of ±5 (CI 1). The overall trend in the asynchronous conditions was decreasing spectral-variance release with increasing bandwidth. An increase in protected bandwidth might have had two effects: 1) reduced the amount of peripheral masking thereby decreased thresholds in the presence of central maskers, and 2) increased similarity between the central C and R maskers (due to the restricted number of unique stimulating electrodes), which in turn would lead to similar detection thresholds in the two masker conditions. These two counteracting effects might explain the general trend seen in the asynchronous conditions.

The observed pattern in the synchronous conditions was more complex and might reflect the ambiguity of the stimuli, in that here temporal cues hampered and spectral-variance cues facilitated signal detection. Listeners CI 3, 4, 5, 6, 7, and 8 did not benefit from increased protected bandwidth. Listener CI 2, however, showed a marked increase in spectral-variance release with protected bandwidth. Interestingly, this listener had relatively narrow peripheral filters (Fig 1.1C, dark blue). The non-linear, peaked function might reflect an optimum for the two counteracting effects of increased protected bandwidth discussed above. Note also that with the ±5-electrode wide protected band variability was largest, again indicating increased difficulty of the task and the possible involvement of central auditory processing.

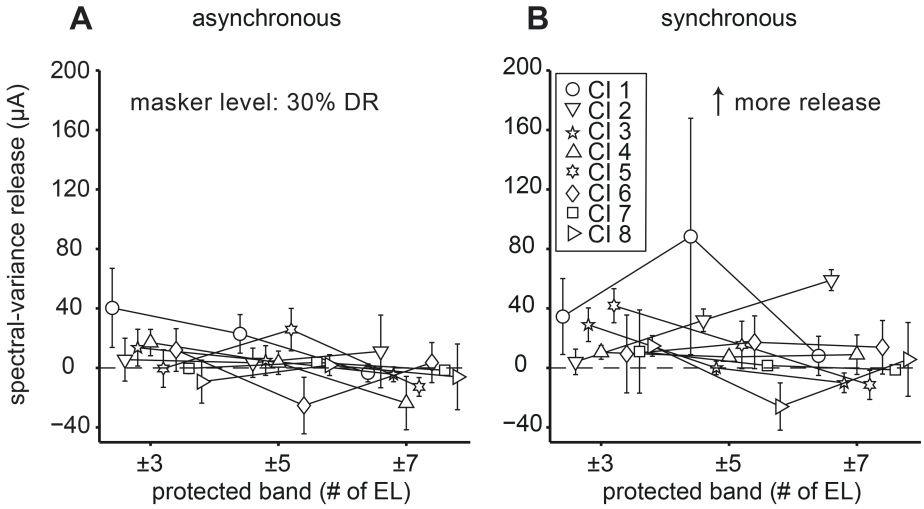

Figure 1.7. Effect of protected bandwidth on spectral-variance release. Data from all three protected band conditions (±3, ±5, ±7 electrodes) at 30% dynamic range (DR) masker level are plotted. **A** Asynchronous conditions. **B** Synchronous conditions. The dashed line at zero demarcates no difference in central (C-R) thresholds as a function of protect band expressed in terms of number of electrodes (# of EL). All other conventions are as in Figure 1.5.

No correlation between spectral-variance release and audiological factors

We performed correlation analyses between spectral-variance release with audiological factors including age, hear loss onset age, deafness duration, years of hearing aid usage and years of CI usage, respectively, and found no correlations. Furthermore, correlation analysis between spectral-variance release and speech recognition with +10 dB signal to noise ratio (Hearing In Noise Test (HINT)) in our sample of eight CI listeners revealed no correlation; a much larger sample size, however, would be necessary to conclude with certainty whether our measures of spectral-variance release correlate with speech-in-noise recognition scores.

EXPERIMENT II: ACOUSTIC SIMULATION OF CI PERIPHERAL AND CENTRAL MASKING RATIONALE

We designed Experiment II to test the hypothesis that broad peripheral filters could degrade the spectral-variance cues, while leaving the temporal cues intact; thus offering a potential explanation for the results observed in CI listeners (Experiment I). We used noise bands to acoustically simulate CI listening to test this hypothesis in five NH listeners. Simulated peripheral filter width was controlled by adjusting the noise bandwidth. We predicted that NH listeners would show elevated R thresholds *re* C thresholds with increasing overlap between noise bands, which would mirror generally reduced spectral-variance release in CI listeners.

EXPERIMENT II. METHODS

Normal hearing listeners

Five NH listeners (three females; age 24-72, mean age 44) were recruited to participate in the simulation experiments. All listeners had normal audiograms with pure tone thresholds below 20 dB HL at low frequencies with the exception of listener NH 3 (age 63) and NH 4 (age 72) whose thresholds were 35 dB HL and 45 dB HL at 8 kHz, respectively and 70 dB HL at 12 kHz, for both listeners.

Acoustic stimuli

We simulated implant listening in NH listeners using 22 noise bands (representing 22 frequency channels) of varying bandwidth centered at logarithmically-spaced frequencies ranging from 0.2-14 kHz in steps of 0.3 octaves. Instead of specifying electrode numbers, burst durations and timings for the HEINRI system, acoustic stimuli were generated in MATLAB, amplified via a sound card (Creative Labs E-MU 0404 USB digital audio system, Creative Technology Ltd., Singapore, 16-bit, 44.1 kHz) and presented monoaurally via calibrated circumaural headphones (HDA-200, Sennheiser electronic GmbH & Co. KG, Wedemark, Germany). To simulate non-overlapping and overlapping CI filters, we tested three noise bandwidth conditions expressed in octaves. Note that we did not correct for edge effects, i.e., we clipped the noise bands at 0.2 and 14 kHz. Therefore, noise bands with high and low center frequencies are asymmetrical and as a result can have bandwidths spanning at most 1.0 octave. The signal was a noise band with a center frequency of 1851 Hz and a protected band corresponding to ±3 center frequencies or 2.0 octaves was used in all three conditions. Both signal and masker were 40-ms noise bursts,

pulsed four times at a rate of 5/s. The same six stimulus conditions used for the electric stimuli were used for acoustic stimuli.

2AFC paradigm for testing NH listeners

To test the NH listeners we used the 2AFC paradigm, stimulus generation routines, and software similar to the ones used in Experiment I. We presented individual masker bands at 60 dB SPL and initially set the signal level to 70 dB SPL. On subsequent repetitions, we set the signal level to 10 dB SPL above the previously tracked threshold level. Large step sizes of 3 dB were used for the first three reversals and small step sizes of 1 dB were used for the next seven reversals. We repeated this measurement for each noise bandwidth condition. Detection thresholds were based on the median of five repetitions in each NH listener except for listener NH 4 (three repetitions). We used the same masker presentation scheme as in the electric experiments with the three overlap conditions presented in random order. Before detecting masked signals, listeners tracked an unmasked threshold for the signal in each overlap condition to provide a baseline against which to compare masked thresholds.

Training

We started training of signal band detection in the presence of asynchronous R maskers with no overlap. Initially, we set the masker level to 40 dB SPL to facilitate detection. After obtaining a threshold at this masker level, training was repeated at increased masker levels (50 and 60 dB SPL) until the threshold tracking curves stabilized (i.e. plateaued after ten reversals).

EXPERIMENT II. RESULTS

Simulated wide peripheral filters degrade spectral-variance cues while leaving temporal cues intact

Fig 1.8 shows the effect of noise bandwidth on signal detection thresholds from the simulations in a similar format as used in Fig 1.3. Detection thresholds were referenced to those measured in an unmasked, signal-only condition (dashed line). For a noise bandwidth of 0.3 octaves (Fig 1.8A), thresholds largely mirrored our previous results with multi-tone maskers (Bremen & Middlebrooks, 2013). Detection thresholds were lower in the asynchronous (red) *re* synchronous (blue) conditions, which demonstrated that central processing of the temporal cues remained intact. Central R maskers tended to yield lower thresholds *re* central C maskers. In contrast to some CI listeners, thresholds for NH listeners in the presence of peripheral maskers were higher than in the corresponding central masker conditions. Also note that as previously reported in the informational masking literature (Kidd et al., 2008) inter-listener variability was large. For example, listener NH 4 experienced little central masking in the asynchronous conditions (Fig 1.8A, C and R). Only listeners NH 1 and NH 3 experienced central masking release in the synchronous condition (Fig 1.8A). On a group level, as noise bandwidth increased: 1) detection thresholds in the presence of central, but not peripheral, maskers increased and 2) inter-listener variability decreased (compare Fig 1.8A-C).

The P thresholds were significantly higher than the R thresholds ($p < 0.05$; Friedman's test using a Bonferroni correction) and the C thresholds ($p < 0.05$; Friedman's test using a Bonferroni correction) (Fig 1.8). In the synchronous conditions, only R thresholds ($p < 0.05$; Friedman's test using a Bonferroni correction) were significantly

higher than the thresholds obtained with P maskers. Note that with increased noise bandwidth the difference between thresholds obtained with R and C maskers decreased notably.

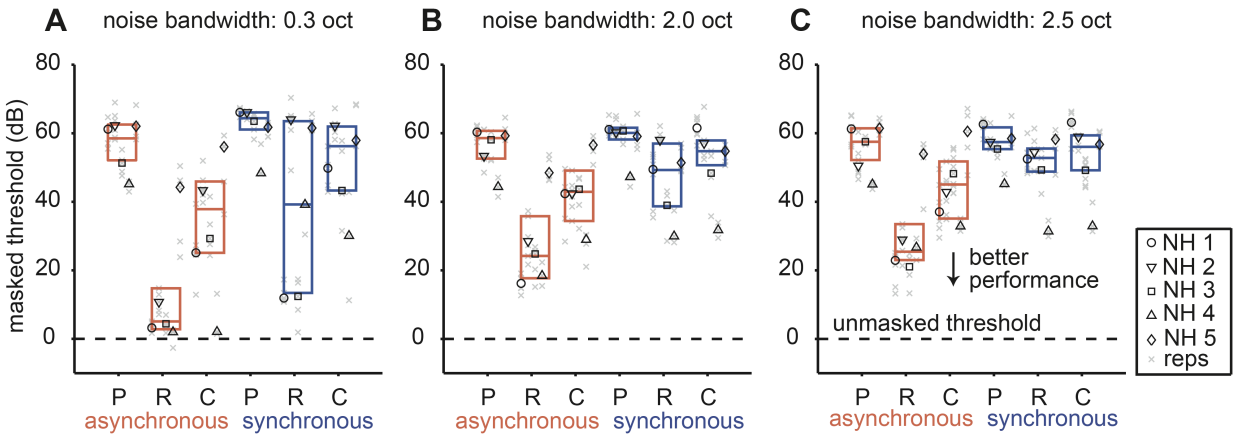

Figure 1.8. Effect of noise bandwidth on signal detection thresholds in CI simulation. Capital letters on abscissa correspond to peripheral (P) and central (C, R) maskers in both asynchronous (red) and synchronous (blue) timing conditions. Detection thresholds re unmasked threshold for the signal presented in quiet (dashed line) is plotted as a function of stimulus. Three different noise bandwidth conditions are shown: **A** ±0.3 **B** ±2.0 and **C** ±2.5 octaves. All other conventions are as in Figure 1.3.

To quantify the effect of noise bandwidth on spectral-variance release we plotted C-R threshold differences and bootstrapped standard deviations (see Methods I) in the asynchronous (Fig 1.9A) and synchronous (Fig 1.9B) conditions. In general, spectral-variance release either stayed constant or decreased across the three bandwidth conditions while intra-subject variability was relative constant across conditions (~10 dB). Individual thresholds, however, could vary considerably with some listeners exhibiting no spectral-variance release and others exhibiting large amounts of release. This observation is in accordance with previous reports and is commonly attributed to different listening strategies, i.e. inter-listener differences in central processing (Neff et al., 1993; Neff & Callaghan, 1988). It is likely that prior to implantation individual CI listeners also would

have employed different central listening strategies. This effect might have influenced detection thresholds in our CI listeners in addition to any effects due to electrical stimulation.

In NH listeners who benefited from the spectral-variance cues, an increase in noise bandwidth decreased the amount of spectral-variance release, e.g. listener NH 3 showed a decrease of ~40 dB from 0.3 to 2.5 octave bandwidth (Fig 1.9B). This seemed to suggest that deteriorated peripheral input, simulated here by increasing overlapping spectra, severely hampered central processing. In relation to CI listeners, the NH data seemed to indicate that wide peripheral filters were one aspect of peripheral encoding that could contribute to the reduced spectral-variance release observed across CI listeners. These acoustic simulations of implant listening seemed to suggest that restricted frequency resolution in the periphery weakened spectral-variance cues but not temporal cues accessible to the central auditory system.

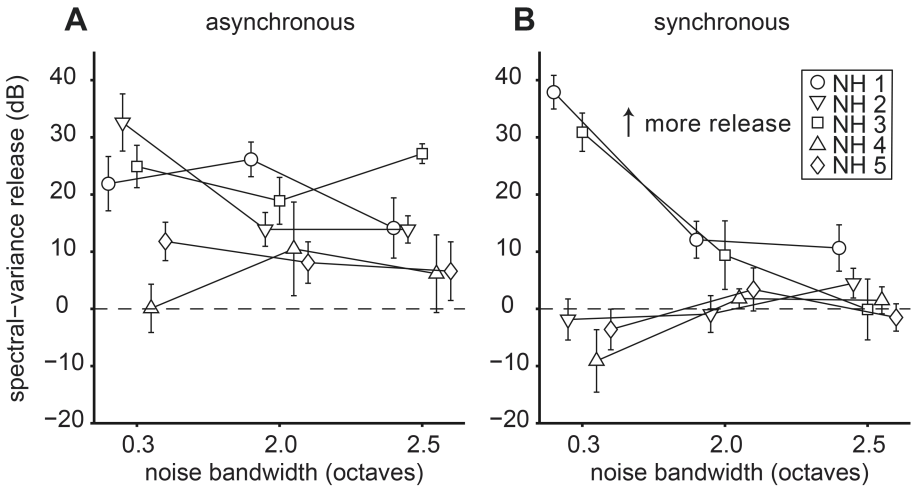

Figure 1.9. Effect of noise bandwidth on spectral-variance release. A Asynchronous conditions. **B** Synchronous conditions. The dashed line at zero demarcates no difference in central (C-R) thresholds as a function of noise bandwidth expressed in octaves. All conventions are as in Figure 1.5.

DISCUSSION

Our findings supported the hypothesis that post-lingually deaf CI listeners retain certain central processing abilities, but these are severely impaired by poor peripheral encoding. We showed that: 1) central processing of timing remained intact in all CI listeners, whereas central processing of spectral-variance seemed to be maintained in only two out of eight CI listeners, and 2) simulating implant listening in NH listeners with normal central processing showed that broad peripheral filters limited the amount of spectral-variance release from central masking.

CI listeners can retain NH-like central auditory processing

All CI listeners could use the temporal cues for signal detection, but not all CI listeners fully benefitted from the spectral-variance cues (Figs 1.4B, 1.5). In particular, listeners CI 1 and CI 7 showed the largest spectral-variance release from central masking. One factor potentially influencing spectral-variance release could have been filter bandwidth. In an attempt to improve spectral resolution, we tested increased protected band conditions (Figs 1.6, 1.7). In the asynchronous conditions, spectral-variance release tended to decrease with increasing protected bandwidth. In the synchronous conditions, however, the observed spectral-variance release pattern was more complex. Increasing the protected band on the one hand further limited the potential for peripheral masking of the signal electrode, but on the other hand decreased the number of electrodes available for the central masking stimuli, i.e. the R masker became more similar to the C masker. Therefore one might conjecture that listeners CI 1 and CI 7 have more perceptually independent channels in comparison to the other CI listeners in our sample, which enabled

better use of spectral-variance cues to segregate the masked signal. Although the results did not reach significance in our small sample of CI listeners, the trend in Fig 1.5 suggested that listeners CI 1 and CI 7 could make use of the spectral-variance cues to gain central masking. Central processing of spectral variance, however, is largely hampered by poor peripheral encoding.

Conversely, spectral resolution as estimated by peripheral filter width does not fully account for central masking release. Listener CI 4, for instance, had extremely narrow filters but did not benefit from spectral-variance cues (Fig 1.1C, green). Conversely, listener CI 1 had relatively narrow filters but showed considerable spectral-variance release (Fig 1.1C, red). We, therefore, considered the possibility that other peripheral factors, such as the uniformity and health of surviving auditory neurons and their proximity to the CI electrode (Fu & Nogaki, 2005) are potential sources of degraded peripheral input which could in turn limit central masking release. By extension, poor peripheral encoding of spectral information hampers central processing of speech. Reduced spectral resolution due to a limited number of perceptual spectral channels and/or channel interactions across electrodes could be responsible for the absence of fine spectro-temporal cues. In turn loss or degradation of these cues may contribute to poor speech understanding in noise, especially in dynamically changing backgrounds in which there are competing speakers or modulated noise (Stickney et al., 2004). Degraded temporal fine structure processing in CI subjects has proven to be detrimental for speech understanding in noise (Stickney et al., 2004).

Evidence from human intra-cranial electrocorticography concur with our behavioral results that CI listeners retain NH-like central auditory processing (Nourski et al., 2013).

Intra-cranial recordings of responses to CI stimulation in a human bilateral CI patient revealed cortical responses seemed quite similar to those obtained in NH, epilepsy patients (Nourski et al., 2013). Latencies of the auditory evoked potential waveform peaks (P_α, N_α, P_β, N_β) in response to 100-Hz clicks in the CI subject were all within the range of latencies seen in 10 NH control participants. Considering how spectral resolution from independent stimulation channels provided by the implant is limited (Fu & Nogaki, 2005), CI listeners depend heavily on temporal envelope information for speech perception.

Simulated CI listening highlights individual differences in central auditory processing

We initially hypothesized that broad peripheral CI filters could fully account for degraded central processing of spectral variance. Indeed, less spectral-variance release with increasing noise bandwidth observed in NH listeners (Experiment II) seemed to support this view. Modeling broad peripheral filters acoustically with noise bands of varying bandwidth, however, could not account for *all* differences observed between acoustic and electric hearing. NH listeners still performed better with 'wider-band' stimuli (i.e. showed spectral-variance release) in comparison to CI listeners with wide peripheral filters. Thus, relative differences in the degree of spectral-variance release suggest differences in central processing abilities and the neuronal representation of relevant cues between the two listener groups. The limited dynamic range in CI listeners might reduce spectral contrast (Loizouet al., 2000). We did not attempt to simulate this factor in the present study. The influence of a limited dynamic range could, for example, be simulated by using a limited number of quantized loudness steps across the full acoustic dynamic range

or by adding additional broadband noises to compress the stimuli into a limited acoustic dynamic range.

Inter-listener variability, generally, tended to be large with central (informational) masking stimuli, i.e. across NH listeners these complex maskers could either strongly elevate or only mildly raise detection thresholds (Neff & Callaghan, 1988). By design, our stimuli contained cues that could either facilitate or hamper signal detection. For example, in the easiest masking condition, asynchronous R, both the temporal and the spectral-variance cues could facilitate signal detection. In this case listeners could potentially reach ceiling performance. Conversely, in the most difficult case (synchronous C) performance could have been close to floor performance. In the extreme cases (floor and ceiling performance), response variability would be small. Conditions in between these two extremes, however, would have led to increased response variability if the listener were capable of accessing all or some of the cues. In contrast to the other CI listeners, listener CI 1 exhibited not only spectral-variance release but also a systematic increase in response variability. Accordingly, we surmise that under the most favorable peripheral encoding conditions, i.e. relative narrow peripheral filters and large dynamic range as seen in listener CI 1, CI listeners retain central processing abilities similar to those of NH listeners. Currently, the origin of inter-listener differences both in CI and NH listeners remains unclear.

Implications for speech perception

Limited spectral resolution and dynamic range distort the internal representation of spectral contrast important for segregating speech from noise (Loizou et al., 2000; Nelson

et al., 1996). Friesen and colleagues (2001) tested recognition of simple sentence material presented at a 5 dB signal-to-noise ratio, and showed that more spectral channels were required in noise compared to in a quiet condition to achieve similar performance. They also demonstrated that most CI listeners are not able to fully utilize the spectral information provided by the number of electrodes used in their implant. Their results align with our findings. Thus, for improving speech-in-noise perception, it seems vital to increase frequency selectivity by e.g. developing new types of auditory prostheses with improved spectral resolution (Middlebrooks & Snyder, 2007) and to increase the dynamic range of CIs by e.g. developing better electro-neural interfaces for current generation implants (Evans et al., 2009; Leake et al., 2011). It is also important to realize that the central auditory system of CI listeners still employs central processing strategies despite the artificial nature of electrical stimulation. With narrow peripheral filters and a large dynamic range, CI listeners might be able to exploit not only temporal cues but also better perceive spectral-variance cues, which are important factors in speech understanding in complex auditory scenes.

Author Contributions

Conceived and designed the experiments: CQP, PB, MML. Performed the experiments: CQP, WS, SY. Analyzed the data: CQP, PB, MML. Contributed reagents/materials/analysis tools: CQP, PB, JCM, FGZ, MML. Wrote the paper: CQP, PB, WS, SY, JCM, FGZ, MML. Designed the software used for psychophysical testing: MML. Subject recruiting for research: CQP.

CHAPTER 2

Acute Effects of Cholinergic Activation via Nicotine Gum on Human Central Auditory Processing

ABSTRACT

Studies in rodent primary auditory cortex (A1) show that systemic nicotine narrows frequency receptive fields and enhances processing within those narrow receptive fields. The enhanced processing included increases in peak amplitudes, decreases in spike onset latency, and decreases in the variability in latency in tone-evoked responses. Thus, we postulated that nicotine in humans enhances related aspects of central auditory processing in line with nicotine-induced improvements in cognition. Experiment I measured tone-in-noise detection at two sound levels to test the hypothesis that nicotine increases central gain. Experiment II involved detecting a temporal gap between two tones of the same (within-channel, WC) or different frequencies (between-channel, BC) to test the hypothesis that nicotine improves temporal acuity. Experiment III used spectral ripples to test the hypothesis that nicotine increases frequency selectivity. Experiment IV measured attention measures based on reaction times and error rates in a selective auditory attention task to test the hypothesis that nicotine enhances processing of the relevant cue while suppressing the irrelevant cue. The study employed a single-blind, randomized, crossover design. Healthy, normal hearing, adults received nicotine (Nicorette 6 mg) or placebo gum on a given session. Subjects performed perceptual tests before and after treatment. Differences between post- and pre-treatments were used to indicate main drug effects. The lack of nicotine effects in tone-in-noise sensitivity, temporal acuity, and frequency resolution likely

reflect maximal performance in healthy, normal hearing subjects with high attentional processing. Variability in BC gap thresholds, however, just significantly decreased with nicotine could reflect improved temporal summation in cortical neurons. Reaction times, error rates, and derivative attention measures appeared unaltered by nicotine. Future study of clinical subject populations with reduced attentional function due to reduced cholinergic activation may show stronger drug effects in challenging perceptual tasks.

INTRODUCTION

During selective attention, the release of endogenous neurotransmitter, acetylcholine, binds to and activates nicotinic acetylcholine receptors (nAChRs) to enhance sensory processing (Gotti et al., 2006; Lee & Dan, 2012). The exogenous drug, nicotine, also targets and binds to nAChRs, presumably "hijacking" the cholinergic contribution to diverse cognitive functions, notably attention (Metherate et al., 2012). Numerous behavioral studies testing a variety of tasks in animals and humans (see Appendix A for a selected review of nicotinic effects on sensory attention) have shown that cognitive functions improve with systemic administration of nicotine or nicotine agonists at nAChRs and worsen with nicotinic antagonists or disease-induced nAChR loss (Levin et al., 2006; Sarter et al., 2009). There is some uncertainty in determining the extent to which nAChRs regulate sensory processing and which sensory-cognitive functions are enhanced by nicotine in humans. The present study aims to promote an understanding of nicotinic effects on diverse human auditory processes including frequency and temporal resolution and auditory discrimination.

In sensory cortices, attention can transiently enhance neural responses to attended stimuli and/or reduce responses to unattended stimuli (Fritz et al., 2003; Miller et al., 1972; Moran & Desimone, 1985). The stimulus filter theory of nicotine hypothesizes that nicotine acts as a 'stimulus filter' to screen out or reduce processing of task-irrelevant stimuli and increase processing of relevant stimuli (Kassel, 1997). This model posits dual processes that 1) reduce cue utilization via attentional narrowing and 2) increase processing capacity to enhance signal-to-noise ratio as more relevant cues are processed with nicotine. Nicotine may also act as a 'stimulus barrier' to block out irrelevant stimuli (Friedman et al., 1974). Given the role of nAChRs in endogenous attention mechanisms, it is reasonable to propose that nicotine may activate nAChRs to exert similar effects on different aspects of auditory processing. Loss of nAChR function following drug-induced nAChR blockade, genetic alteration in knockout mice and disease-induced loss as in Alzheimer's Disease can impair sensory processing (Levin et al., 2006; Sarter et al., 2009). Pharmaceutical companies have developed nicotine-based drugs to treat attention/cognitive deficits accompanying attention deficit disorders and Alzheimer's Disease (Hurst et al., 2013), however, no drug treatment has targeted attention-deficits in auditory processing disorders. The present study investigates the effects of oral nicotine on diverse aspects of auditory function to build a foundation for long-term research evaluating the therapeutic value of using nicotine-related drugs to enhance attention in treating central auditory processing deficits. We postulated nicotine enhances related aspects of human central auditory processing in line with general improvements in learning, working memory, and attention in different species (Levin et al., 2006; Sarter et al., 2009).

In a more direct study of nicotine's effects on human auditory processing, systemic nicotine delivered to non-smokers via transdermal nicotine patch seemed to affect central but not peripheral auditory responses. Medial olivocochlear efferent synapses onto outer hair cells in the periphery are nicotinic and, presumably, modulate sound amplification that can aid auditory attention. Otoacoustic emissions, "echos" produced by the outer hair cells, access cochlear amplification. Transdermal nicotine produced no effect on otoacoustic emissions but did affect auditory evoked responses including middle latency and long-late latency responses (Harkrider & Champlin, 2001a, 2001b). These studies selected nonsmokers to avoid effects associated with chronic nicotine exposure and consequently were limited to low-dose patch (release 7 mg/24 h and remained in place for 4 h) because of adverse side effects (e,g., nausea). The patch produces modest changes (both increases and decreases) in latency and amplitude of specific evoked potential components associated with auditory nuclei throughout the auditory pathway, including increase amplitude and decrease latency of long-latency (forebrain) potentials. In both smokers and non-smokers, nicotinic increase in consonant-vowel discrimination in noise as measured behaviorally and electrophysiological support an auditory gating role for nicotine (Harkrider & Hedrick, 2005). The lack of nicotine effect on otoacoustic emissions in the cochlea support a central rather than peripheral effect of nicotine.

Studies in rodent primary A1 auditory cortex show that systemic nicotine narrows frequency receptive fields and enhances processing within those narrowed receptive fields (Intskirveli & Metherate, 2012; Metherate et al., 2012). We interpreted this finding as a potential increase in perceptual frequency selectivity and central gain, i.e., signal amplification in the brain. Nicotinic enhances neuronal responses to characteristic

frequencies and suppresses responses to non-characteristic frequencies (Intskirveli & Metherate, 2012), indicating sharper frequency tuning potentially relevant in attention processes. Similarly, auditory cortex of humans and monkeys (without nicotine) simultaneously increase cortical processing (gain) of attended acoustic stimuli and narrow frequency tuning (Lakatos et al., 2013; Okamoto et al., 2007). Hence, we think attending to behaviorally relevant sensory cues depend on nAChR, which enhance cue-evoked responses and similar effects result from exogenous nicotine administration (Metherate et al., 2012).

In rodent A1 neurons, systemic nicotine increases the peak amplitude of local field potentials and decreases spike onset latency and variability latency of tone-evoked responses (Kawai et al., 2007). Nicotine binding to nAChRs in the initial portion of the auditory thalamocortical pathway causes spikes to reach cortex more rapidly and synchronously due to increased temporal summation which in turn increases response amplitudes (Kawai et al., 2007). We reasoned this robust cortical effect changes perceptual temporal processing. Thus, we used gap stimuli inspired by (Phillips et al., 1997) to measure temporal acuity by the central auditory system (Musiek et al., 2005). A stimulus containing a silent gap within a frequency (within-channel, WC) or between different frequencies (between-channel, BC), presumably, excites overlapping or non-overlapping neural populations, respectively, in turn activating one or multiple perceptual channels. In children with auditory processing disorders, WC gap in noise processing were similar to controls (3.5 – 5.3 ms) but BC gap processing showed impairment (53 - 180 ms) compared to control (24 - 80 ms) (Phillips et al., 2010). Children with processing disorders might show smaller differences between WC and BC due to wider perceptual channels. Thus, we

became interested in whether nicotine affects temporal gap detection, especially of BC gaps considering more central effects of the drug.

To more directly test nicotine effects on auditory attention, we used the test of attention in listening (TAIL; Zhang et al., 2012), which requires listeners to make same/ different judgments when directing attention to a relevant stimulus cue (e.g., frequency or location- ear of sound presentation) and ignoring the irrelevant stimulus cue (e.g., location or frequency). Zhang et al., 2012 describes relationships between TAIL and brain attention networks. Briefly, in the TAIL, involuntary orientating attention to irrelevant cues would reflect inhibition of the ventral network (Corbetta et al., 2008), i.e., suppressing competition of irrelevant stimuli for attention resources. The anterior cingulate cortex and related brain structures appear to mediate executive control in monitoring conflicting information and recruiting lateral frontal areas to resolve conflicts (Botvinick et al., 2004; Petersen & Posner, 2012). In nonsmokers, nicotine gum (2 mg) increases speed for resolving conflicting word/color information in the Stroop test (Provost & Woodward, 1991). Transdermal nicotine (7 mg) improved some measures of sustained attention in the low attention group but impaired working memory in the high attention group, which suggests that nicotine tends to optimize rather than improve performance on cognitive tasks (Poltavski & Petros, 2006). By the attention allocation model, nicotine acts as a stimulus filter or stimulus enhancer, depending on task conditions (Kassel, 1997). An expected role for nicotine may be to increase or decrease recruitment of attention-associated brain areas according to task demands (Smucny et al., 2015).

The present study investigated acute effects of nicotine on diverse auditory tests with varying task demand and attention load. Experiment I measured tone-in-noise

detection at two sound levels to test the hypothesis that nicotine increases central gain i.e., nicotine amplifies signals in the brain. Experiment II involved detecting a temporal gap between two tones of the same frequency (within-channel, WC) or different frequencies (between-channel, BC) to test the hypothesis that nicotine improves temporal acuity. Experiment III used spectral ripples to test the hypothesis that nicotine increases frequency selectivity. Experiment IV employed an auditory attention task requiring discrimination of two tones using frequency or location (ear of sound presentation) cues. We hypothesized that nicotine increases auditory and attention-mediated processing by increasing (1) central gain, (2) temporal acuity, (3) frequency selectivity, and (4) differential patterns of attention contributions across tasks.

METHODS

Ethics statement

After receiving complete explanation of the study, all subjects gave written informed consent to participate in experiments approved by the University of California Irvine's Institutional Review Board. All subjects received compensation for hourly participation.

Subjects

Eighteen individuals were recruited and a total of 14 subjects participated in the experiments (age range: 18- 27 years, mean ± sd: 21±2.6 y; 9 males; 12 right-handed). An online survey facilitated initial subject screening to ensure no known hearing dysfunction, medical or mental health illness (including drug dependency, diabetes mellitus, renal failure, cardiovascular disease, neurological disease, psychiatric disorder, central nervous

system disorder, or regular use of prescription medication (excluding oral contraceptives)), and low nicotine dependence, use, and exposure (score average of 0-2 out of 10 maximum on the Fagerström index of smoking dependency (Bramer & Kallungal, 2003; Heatherton et al., 1991; see Appendix B & C). Twelve subjects had no smoking history (i.e., smoked no more than 100 cigarettes in their lifetime and none in the past year; Knott et al., 2014) and two smoked socially (i.e., smoked no more than 1 cigarette per week or 4 per month; S1 and S8). Chronic smokers were excluded from the study to avoid confounding effects of nicotine- withdrawal on cognitive effects i.e., measuring potential reversal of withdrawal-induced attention deficits opposed to increase of auditory attention processes. Subject recruitment focused on individuals with limited nicotine-exposure because the long-term goal aims to determine nicotinic effects in patients having central auditory processing disorders and who are likely to be nonsmokers. Most subjects had never smoked cigarettes or used nicotine during the last 2 years. To avoid chemical interactions, all subjects were asked to abstain from the following prior to testing: (1) drug use for ≥3 days (2) alcohol consumption for 24 h (3) food consumption ≥1 h. To avoid caffeine withdrawal in regular caffeine-consumers, one 1/2 cup of caffeine-containing beverage ≥1 h was permitted (Lawrence et al., 2002). At the beginning of each session, female subjects took a pregnancy test in a private bathroom in the laboratory; the negative results were required for continued participation. Eligible subjects had audibility ≤20 dB HL (decibels Hearing Level) at octave frequencies between 0.125 and 12 kHz, bilaterally. Data from four individuals were excluded from further analysis due to elevated audibility (1 subject) or incomplete treatment sessions (3 subjects) leaving 14 subjects whose data were analyzed. Subjects were given the option of participating in more than one experiment, enabling intra-subject

comparisons across tasks. Intra-subject correlation analyses were possible with ≥8 subjects who participated in all four experiments (S1, S10-16). Ten subjects completed Experiment I (S1, S8, S10, S11-16, S19); eleven subjects completed Experiment II (S1, S5, S8, S10-16, S19); nine subjects completed Experiment III (S1, S8, S10-16); twelve subjects completed Experiment IV: TAIL (S1, S2, S10-16, S18, S19). Table 2.1 describes subjects' experimental details.

Subject	Sex	Age	Handedness	Expt I: TIN central gain	Expt II: TGD temporal acuity	Expt III: SMRT frequency resolution	Expt IV: TAIL auditory attention
1	F	23	R	X	X	X	X
2	F	27	R	--	--	--	X
5	M	20	R	--	X	--	--
8	M	25	R	X	X	X	--
10	M	20	L	X	X	X	X
11	M	20	L	X	X	X	X
12	M	18	R	X	X	X	X
13	M	21	R	X	X	X	X
14	M	18	R	X	X	X	X
15	M	20	R	X	X	X	X
16	M	19	R	X	X	X	X
18	F	19	R	--	--	--	X
19	F	18	R	X	X	--	X
20	F	19	R	--	--	--	X
total	5F: 9M	21±2.6	12R: 2L	10	11	9	12

Table 2.1 Subject's experimental details.
Eight out of 14 subjects participated in all four experiments (TIN, TGD, SMRT, and TAIL). Assignment of treatment sequence (nicotine-placebo or placebo-nicotine) was random and the order of drug administration was counterbalanced over subjects. Gray boxes denote subjects who tested with nicotine first and white boxes denote subjects who tested with placebo first (see Fig 2.1, Group A and B). Participation is denoted with an 'X' and incomplete/ non-participation as '-- '. Age across subjects is expressed as mean ± sd. TIN:

Tone-in-noise detection; TGD: Temporal gap detection; SMRT: spectral-temporally modulated ripples test; TAIL: Test of attention in listening.

Study design

Sessions occurred between 8:30 a.m. and 2:00 p.m., with the majority starting before noon and taking place during a consistent time across sessions so as to regulate arousal levels and to avoid confounding attentional effects (Fig 2.1). At least one day preceding each session, a text message and/or email reminded subjects of abstinence instructions and compliance was confirmed verbally before testing commenced. All experiments took place in a double-walled, sound-attenuated sound booth. Audiograms were measured during the first session. One of four experiments [tone-in-noise detection (TIN); temporal gap detection (TGD), spectral-temporally modulated ripples test (SMRT), test of attention in listening (TAIL)] was tested (TIN and TGD were tested consecutively during the same session). Subjects participated in a minimum of two treatment (nicotine and placebo) sessions, with the possibility of completing all four experiments within 6 sessions. After pre-treatment testing, subjects received either nicotine or placebo gum in a randomized design. The protocol was repeated with the alternate treatment (nicotine or placebo), adhering to a single-blind intra-subject design. Treatment sessions were separated by ≥48 h to allow for treatment clearance (Fig 2.1). Pulse rate measured via pulse oximetry (Choice MMed America Co; Thiel & Fink, 2007), mood, and side effects (Harkrider & Hedrick, 2005; Parrott et al., 1996; see Appendix D) were monitored before and after treatment (Lawrence et al., 2002). Pulse rate measurements from four subjects (S5, S8, S10, S11) were lost.

Drug administration

Nicotine was delivered in the form of two pieces of mint-flavored polacrilex gum (4mg and 2mg; Nicorette®, Johnson & Johnson, Inc). The total 6-mg dose produces a nicotine plasma concentration of ~15 – 30 ng/ml, which is the approximate blood concentration after smoking one, medium nicotine yield, cigarette (Hukkanen et al., 2005). This dose was selected based on previous studies with non-smokers showing drug tolerance without any significant adverse side effects resulting in terminated participation (Knott et al., 2014a; Knott et al., 2014b). Furthermore, our pilot testing showed no behavioral effects with 2 and 4 mg nicotine, which also justified the increased dosage. The placebo consisted of two pieces of commercially available mint-flavored gum (Eclipse®), resembling the nicotine gum in size, shape, color, and texture. Subjects wore a blindfold during treatment administration to mask any potential visual differences between placebo and nicotine gums. A drop of Tabasco sauce was added to each gum piece to disguise taste bias (Thiel & Fink, 2007). To regulate drug administration and minimize side effects, subjects followed manufacturer guidelines to chew the gums for 25 m, biting twice per minute and 'parking' the gum between teeth and cheek between bites when cued by an auditory signal. Following 25 m and prior to blind fold removal, subjects removed the treatment gum and chewed a commercially available, cinnamon-flavored gum for 2 m at the same pace as before to mask any remaining taste differences between treatments (Knott et al., 2014). The "wash" method disguised treatment in 7 out of 10 subjects who participated in multiple experiments and 30% of the time (9 out 32 times polled). In some subjects, changes in mood and/or side effects symptoms could have biased the treatment administered. Post-treatment testing began 30 m from the beginning of treatment

administration for the reason that oral nicotine exhibits peak blood nicotine concentrations 30 m after nicotine gum chewing (Hukkanen et al., 2005). All experiment protocols could be completed in 30-60 m, which is well within the time course of 120 m nicotine elimination half life (Hukkanen et al., 2005). The order of nicotine vs. placebo administration was counterbalanced over subjects.

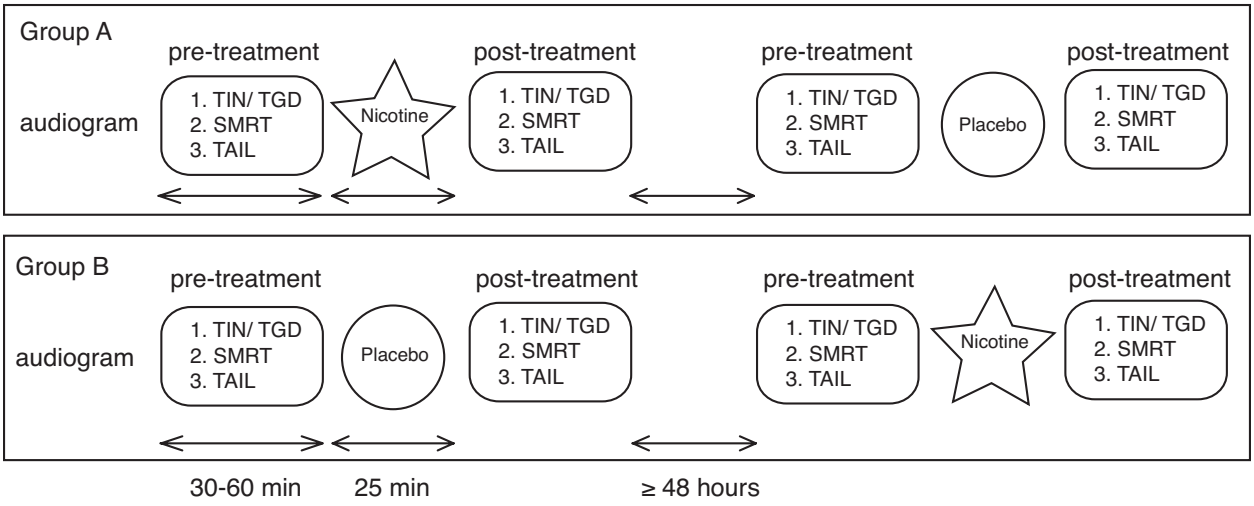

Figure 2.1 Study design.
All four experiments (TIN, TGD, SMRT, and TAIL) could be completed in 6 experimental sessions (TIN and TGD were tested during the same session). Subjects participated in a minimum of two treatment (nicotine and placebo) sessions during the same time window. Audiograms were measured during the first session. After pre-treatment testing, subjects received either nicotine (6 mg) or placebo gum and instructed to bite the gum twice every minute for 25 m (to minimize side effects) in a randomized design. The protocol was repeated with the alternate treatment (nicotine or placebo) during the subsequent session adhering to a single-blind intra-subject design. Pulse oximetry and mood/ side effects were monitored before and after each treatment. Administered nicotine treatment is indicated as stars and placebo treatment as circles. Treatment sessions were separated by ≥48 h to allow for treatment clearance. Subjects who participated of all four experiments completed experiments in order I- IV. TIN: Tone-in-noise detection; TGD: Temporal gap detection; SMRT: spectral-temporally modulated ripples test; TAIL: Test of attention in listening

Stimuli

All acoustic stimuli were generated in MATLAB (The Mathworks, Natick, MA), amplified via a sound card (Creative Labs E-MU 0404 USB digital audio system, Creative

Technology Ltd., Singapore, 16-bit, 44.1 kHz) and presented monoaurally (binaurally in Experiment IV) through calibrated circumaural headphones (HDA-200, Sennheiser electronic GmbH & Co. KG, Wedemark, Germany). Experiment I-III used adaptive procedures (3-alternative forced choice) to measure threshold for tone-in-noise sensitivity, just-noticeable-difference in gap between tones, and threshold for frequency discrimination. Experiment IV involved same/different discrimination of two tones that could differ in frequency and/or ear of sound presentation as subjects attended to pitch or ear and ignored the other parameter.

Tone-in-noise

The target interval presented a tone concurrently with noise whereas the reference interval presented noise alone. Identical noise bands were used in target and reference intervals. The overall duration of each stimulus interval and inter-stimulus interval remained constant at 500 ms. A 2- or 4-kHz sinusoid was shaped with 2.0-ms linear rise-fall times. The noise band consisted of pink noise, band passed with center of 2828 Hz and width of 5-octaves with a -3 dB/ octave slope, fixed at 50 dB SPL. A pink spectrum in the noise passband was chosen because in pilot experiments, pink noise could best reduce acoustic transient gating cues. Low frequencies stimulate a larger area of the basilar membrane and therefore would require more acoustic energy to mask the low frequency region. Pink noise contributes more masking energy to the low than high frequency regions of the cochlea to reduce spectral splatter. The reference stimulus contained noise alone. Experiment I presented tone components at 45 and 70 dB SPL (respectively, ~10 and ~40 sensation level: dB *re* the subject's threshold for that stimulus) summed with a pink noise

component designed to minimize spectral splatter. In total, four tone-in-noise conditions were tested: 2 kHz at 45 and 70 dB SPL, and 4 kHz at 45 and 70 dB SPL.

Temporal gap

Gap stimuli were inspired by narrowband noise stimuli designed by Phillips et al., 1997, however, we designed tonal stimuli to test effects on more restricted cochlear excitation representing the target. Each target stimulus consisted of a pair of components so-called "markers" separated by a gap varying systematically in duration (Fig 2.2A, 10-ms gap shown). The amplitude envelope for the leading and lagging markers bounding the temporal gap was shaped with 2.0-ms linear rise-fall times, including those defining the gap. The gap was inserted into the center of a fixed 500-ms stimulus interval. The markers contained 2- or 4- kHz tones shaped with the envelopes. The lagging marker contained a 2-kHz sinusoid and the leading marker contained either a 2- or 4-kHz sinusoid to create the within-channel (WC) or between-channel (BC) gap stimulus, respectively (Fig 2.3). BC conditions used high-to-low frequency markers (4:2 kHz) opposed to low-to-high frequency markers (2:4 kHz) because the former produces higher gap thresholds by ~1-2 ms (Heinrich et al., 2004) capable of decreasing with nicotine. The summed envelopes produced a gap stimulus with two tonal markers. Initial gap durations could be as long as 100 ms (WC) and 250 ms (BC). The overall duration of each stimulus interval remained constant to avoid presenting duration cues as the gap changed, therefore marker duration could range from 125-250 ms. Each stimulus was separated by a 500-ms inter-stimulus interval. The duration of each gap was specified by the time between the -6 dB point at the end of the leading marker envelope and the equivalent point at the beginning of the lagging marker envelope (Fig 2.2A, B, C). The reference stimulus contained a "0 ms" gap in which

the -6 dB point of the leading marker envelope offset overlapped at the equivalent point of the lagging marker envelope onset to create a perceived, uninterrupted stimulus (Fig 2.2D).

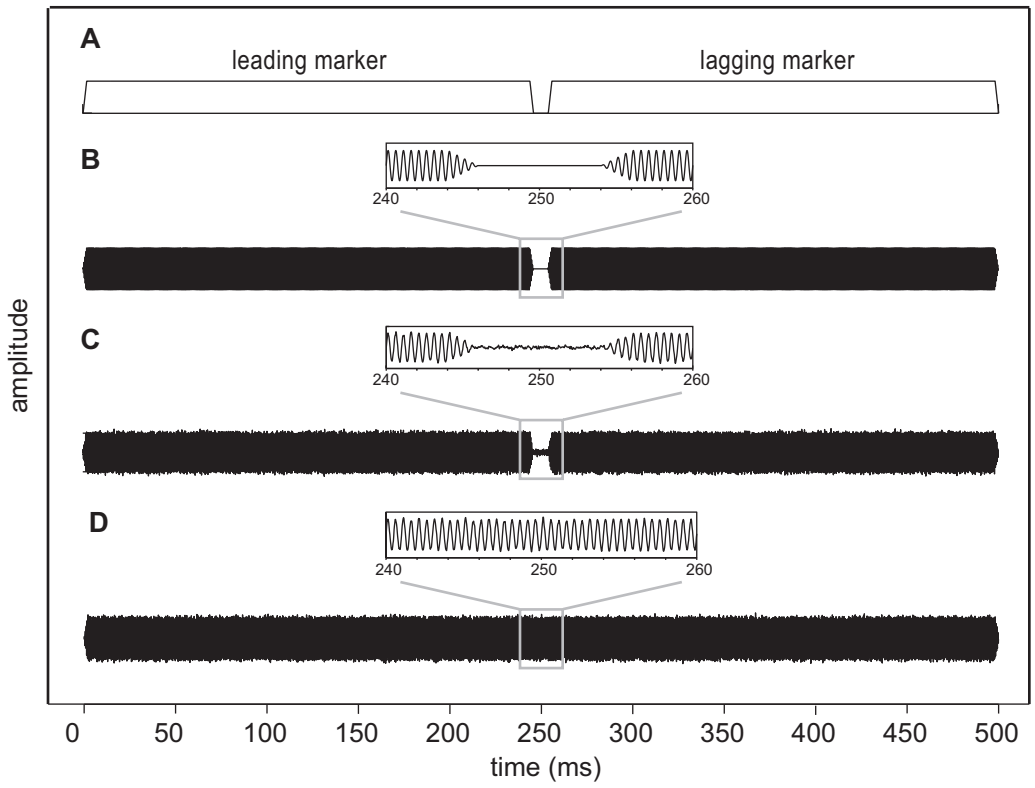

Figure 2.2 Temporal gap stimuli.
A The envelopes of the leading and lagging markers separated by a 10 ms gap. **B** The leading and lagging markers contained 2- or 4- kHz tones shaped with envelopes with 2.0-ms linear rise-fall times, including those defining the gap. The summed envelopes produce two tonal markers bounding a gap. **C** Target stimulus containing a 10-ms gap partially filled with pink noise to reduce spectral splatter arising from transient gating cues (acoustic smearing). **D** Reference stimulus containing a 0-ms gap. The inserts show an expanded view of a 20-ms window centered in each stimulus. See text for details.

The reference stimulus ("0 ms" gap) used in gap detection was identical to the target stimulus used in tone-in-noise detection (Fig 2.2D). Tone components were presented at 45 and 70 dB SPL (~10 and ~40 dB sensation level as in Experiment I) and summed with a 50 dB SPL pink noise component (Fig 2.2C). Two sound levels were chosen with the intention

of varying the task difficulty and increasing cognitive load at lower sound levels. Pink noise, as previously described, was designed to minimize spectral splatter. Although, moderate level masking energy partially filled the temporal gap (Fig 2.2C) this did not interfere with subjects' ability to perform gap detection between the tonal components. BC and WC conditions were tested at two sound levels yielding four total gap conditions: BC45, BC70, WC45, WC70.

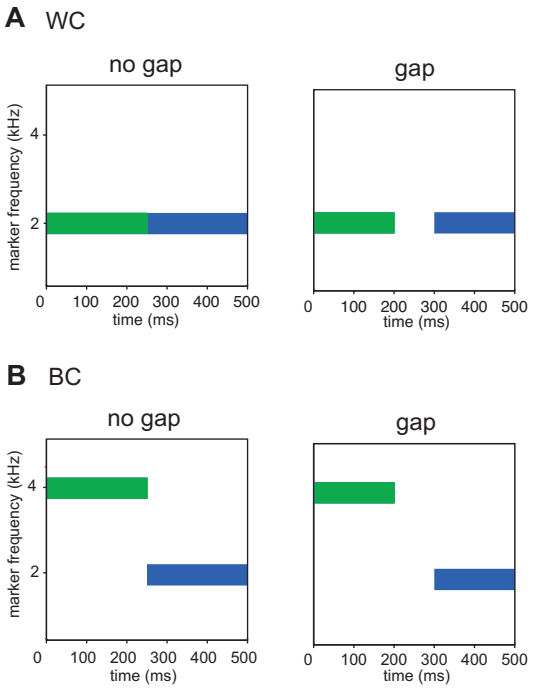

Figure 2.3 Between- and within-channel gap conditions.
Leading and lagging markers are represented by green and blue bars, respectively. **A** The within-channel (WC) gap condition has identical marker frequencies (2:2 kHz). **B** The between-channel (BC) gap condition has different marker frequencies (4:2 kHz). A reference stimulus with no gap (0 ms) and a target stimulus with a gap (100-ms) are shown for each gap condition.

Spectral ripples

The ripple stimuli for testing frequency resolution were developed by Aronoff & Landsberger, 2013. A spectrally rippled stimulus can have amplitude modulation in the frequency domain. The task developed by Aronoff and Landsberger uses a spectral ripple with a modulation phase that drifts with time. Each stimulus was 500 ms in duration,

including 100 ms onset and offset ramps. The stimuli were generated using a non-harmonic tone complex with 202 equal amplitude pure-tone frequency components, separated by 0.03 octave between 100-6400 Hz. The amplitudes of the pure tone components were modulated by a sine function based on the following equation:

$$S(t) = \sum_{i=1}^{202} P(i) \times \left(\left| D \times \sin\left[\frac{(i \times RD \times \pi)}{33.333} + (RR \times \pi \times t) + \varphi \right] \right| + D \right), \qquad (1)$$

where S is the ripple stimulus, P is the pure tone amplitude with index i (100 Hz for i = 1, 102.1 Hz for i = 2, etc.), t is time, RD is the ripple density defined by the number of ripples per octave (RPO), φ determines the phase of the ripple at stimulus onset, RR is the ripple repetition rate (number of times the ripple pattern repeats each second), and D scales the modulation depth of each ripple. To test frequency resolution, only RD and φ varied across stimuli (alternately varying RR instead of RD would enable testing temporal resolution). The spectral ripple stimuli were presented at 45 dB SPL to increase the difficulty level and attention demand of the task to more closely match the low sound level tested with tone-in-noise and gap detection.

Attention in listening

The tonal stimuli in the TAIL were generated as described by Zhang et al., 2012. Briefly, two tone frequencies were drawn randomly between 476–6188 Hz with the constraint that any two frequencies had to differ by ≥2.1 equivalent rectangular bandwidths, producing a perceptual difference larger than the frequency discriminability in all subjects to avoid frequency confusion. In all conditions, tone level varied between 70

to 85 dB SPL and tone duration varied between 100 - 300 ms. The silent interval between the two tones was fixed at 300 ms or randomized between 150 - 450 ms.

Procedures

Experiment I-III used a three-interval, forced choice, adaptive paradigm to present in random order two intervals containing a reference stimulus and one interval containing a target stimulus, varying systematically in the dependent variable (i.e., tone level, gap duration, or ripple density). Subjects were instructed to select the "different" sound after listening to each stimulus play sequentially and clicking via computer mouse one of three buttons corresponding to the target stimulus displayed on a graphic user interface. Subjects took breaks after every 10-15 m of testing. In all four experiments, subjects were instructed to respond as quickly and accurately as possible to engage a more attentive brain state.

Experiment I: Tone-in-noise detection

Thresholds for detecting a 2- or 4- kHz tone in noise presented at two sound levels were used to access central gain with nicotine. We predicted nicotine increases central gain to enable finer detection of stimuli at lower levels, i.e., lower sound level required for detecting a tone in noise. We selected two sound levels, 45 dB SPL and 70 dB SPL, representing two sensation levels (dB above threshold), ~10 dB and ~ 40 dB, in order to see potential changes in central gain. Two reference intervals contained the masker alone and one target interval contained the tone embedded in noise. Subjects were to select the target interval in which they perceived a tone-in-noise. Adaptively tracked thresholds

terminated after reaching 10 reversal points (sign changes) using a 2-down, 1-up procedure, which measures 71% correct performance (Levitt, 1971). After two successive correct responses, the level of the tone decreased by -5 dB for the first 3 reversals and -2 dB thereafter. After one incorrect response, the tone level increased by the same step sizes. The calculated tone-in-noise threshold averaged the last 6 out of 10 trials that produced reversals in the adaptive step.

In practice sessions prior to pre-treatment and post-treatment testing, each subject repeated the four tone-in-noise conditions until thresholds per condition differed by <3 dB before pre-treatment testing. During experiments, four threshold measurements (~5 m each) were made before and after treatment.

Experiment II: Temporal gap detection

Thresholds for detecting a temporal gap between 2:2 kHz (WC) and 4:2 kHz (BC) presented at two sound levels were used to access temporal acuity with nicotine. We predicted nicotine increases gap detection at lower levels, i.e., shorter gap (ms) required for detecting a temporal interruption in a low level stimulus. Two reference intervals contained the "0 ms" gap and one target interval contained two tonal markers bounding a gap varying systematically (Fig 2.3). Subjects were to select the target interval in which they perceived a temporal gap between two tones. As in Experiment I, threshold tracks terminated after 10 reversal points using a 2-down, 1-up procedure measuring 71% correct performance (Levitt, 1971). After two successive correct responses, the duration of the gap in the target stimulus decreased by a factor of 2. After one incorrect response, the

size of the gap increased by the same factor. The calculated gap threshold averaged the last 6 trials that produced reversals in the adaptive step.

All subjects practiced each of the four gap conditions until thresholds plateaued before pre-treatment testing. WC conditions required shorter learning curves and associated with little variance in gap thresholds within subjects. BC conditions required longer learning curves and associated with larger variance in gap thresholds within subjects (Phillips et al., 1997). Our pilot experiments showed that the 6 subjects who trained for three 1-h sessions had similar BC thresholds across all sessions (data not shown) and performed similarly as subjects who trained for only ~1 h before pre-treatment testing. Therefore, training was pared to 40-60 m preceding pre-treatment testing. Each condition was tested 1-3 times (~5 m each) pre- and post-treatment.

Experiment III: Spectral ripple discrimination

The spectral-temporally modulated ripple test (SMRT) adjusted adaptively ripple density of the target stimulus until the subject could not distinguish between the reference and the target stimuli to measure dynamic spectral resolution in terms of ripples per octave. Two intervals contained the reference stimulus (20 ripples per octave) and one interval contained the target stimulus. The target stimulus initially had 0.5 ripples per octave and decreased or increased by 0.2 ripples per octave after each correct or incorrect response, respectively (1-up/1-down adaptive procedure measures 50% correct performance). Randomly selected φ for the target and reference stimulus, separately, could take one of four values: 0, $\pi/2$, π, and $3\pi/2$. The test terminated after 10 reversals and

calculated thresholds based on the average of the last 6 reversals. Modulation depth (D) was set to 20 Hz and ripple repetition rate (RR) was set to 5 Hz.

All subjects practiced ripple discrimination 1-6 times until performance plateaued (<3 ripples per octave change across measurements) before pre-treatment testing. Measurements were repeated 3 times (~5 m each) before and after treatment.

Experiment IV: Attention in listening

The test of attention in listening (TAIL) presented sequentially in each trial two clearly audible tones either of the same or different frequencies or ears, respectively. In 3 testing conditions, tone frequency and location (ear of sound presentation) were manipulated (Zhang et al., 2012). For ease of comparing our TAIL results to the published results, we used the same convention that Zhang and colleagues used for labeling conditions. In the FL condition, frequency was the task-relevant cue and location was the distracting cue. Subjects were asked to press one of two buttons on a custom-made, USB-interface button box as quickly and accurately as possible to indicate whether the two tones were the same or different pitch. In the LF condition, location was the task-relevant cue and frequency was the distracting cue. Here, subjects indicated whether the two tones were presented to the same or different ears. In the Control condition, neither frequency nor location was task-relevant and subjects selectively press one of the two buttons as soon as they hear the second tone. Condition order was randomized across subjects. Each condition had 40 trials and corresponding instructions appeared on the computer screen at the beginning of each test to indicate test condition and avoid task confusion. All subjects used their dominant hand to press the response button.

Before testing each experimental condition, subjects completed five practice trials with the option to repeat the practice as many times as needed to become familiar with the current task. Three conditions were tested with fixed or a randomized silent interval between the two tones. In total, the six conditions took ~30 m to complete. Our analyses focused on the three fixed-interval conditions as the three randomized conditions produced reaction times with similar means but larger variance.

Data analysis and statistics

Individual pairwise t-tests with Bonferroni corrections for multiple comparisons (Schechter, 2004) were used to test significance of post-pre differences in each experiment for treatment effects (e.g., BC45 vs. WC45). We selected this approach to provide an unbiased estimate of treatment effect, assuming no carryover effects (e.g., learning effects carried over from one experimental condition to another; residue effects of the first treatment affecting outcome of the new treatment). Separately analyzing data from Group A (nicotine-placebo) and Group B (placebo-nicotine) revealed non-significant drug effects (all comparisons in the four experiments, $p > 0.01$), which justified further analyses grouping all subjects. To facilitate intra-subject comparisons across experiments all figures used the same subject color code in that different colors denote different subjects and each color corresponds to the same individual across figures.

Reaction times of correct trials served as the primary measure of performance in Experiment IV and were analyzed in similar ways as in Zhang et al., 2012. Reaction times longer than 2 s or shorter than 100 ms, which suggested lapsed attention, interrupted

performance or premature responses, were excluded (~20% trials) from calculations. The Control condition allowed anticipated responses to the second tone and approximately 18% of reaction times fell below the 100-ms criterion. Baseline reaction time per TAIL condition was calculated using the trials in which the two tones were the same in both location and frequency. Involuntary orientation indicates the influence of the task irrelevant cue(s) and quantified the difference between the different and same trials for that (distracting) cue. Conflict resolution indicates the frequency by location interaction and quantified the difference between incongruent (same in one cue and different in the other cue) and congruent (same or different in both cues) trials. A linear regression with a least-squares criterion was used to assess significant correlations between auditory attention effects (reaction times) and auditory processing (tone-in-noise threshold, gap threshold, and ripples per octave).

All statistical analyses were performed in MATLAB.

RESULTS

Pulse oximetry

Nicotine increased significantly the pulse rate (n=10 subjects; mean ± sem across subjects; pre-placebo: 72.3 ± 1.8; post-placebo: 72.5 ± 1.6; pre-nicotine: 73.2 ± 1.5; post-nicotine: 80.5 ± 1.2, pre-nicotine vs. post-nicotine, $p << 0.01$, consistent with other reports that have also tested oral nicotine (Smucny et al., 2015; Thiel & Fink, 2007). This positive control experiment provides evidence that a concentration of nicotine had entered the bloodstream (and brain) before post-treatment testing.

Mood changes and side effects

All subjects provided subjective ratings on a 9-bipolar categories mood scale and a 5-point side effects scale pre- and post- treatment. Ratings were averaged across all four experiments for comparison of pre- to post- treatment effects. Nicotine increased significantly mood ratings of energy (p = 0.01), contentedness (p = 0.03), and focus (p = 0.03). This result differs from previously reported use of a lower dosage, 2 mg oral nicotine, which had no significant effect on alertness, contentedness, or calmness (Thiel & Fink, 2007). Placebo increased significantly ratings of relaxation (p = 0.01), calmness (p = 0.01), energy ($p << 0.001$), alertness ($p << 0.001$), and hunger (p = 0.03). Post-nicotine treatment, subjects rated side effect symptoms as high as 3/5 (jittery, dull headache) on a scale from 1 = none (no symptoms) to 5 = severe (jittery, dull or pounding headache, nausea, vomiting) (Harkrider & Hedrick, 2005). Side effects increased significantly with nicotine ($p < 0.0001$). Objective pulse rate increase after oral nicotine delivery relates to subjective ratings increase and further support nicotine entry into the bloodstream and brain before post-treatment testing.

Experiment I: Tone-in-noise detection

Fig 2.4 shows performance for detecting a tone-in-noise (TIN threshold) in 10 subjects (data from S5 have been excluded due to incomplete testing of the 4 kHz-conditions). Fig 2.4A shows scatter plots comparing post vs pre (placebo or nicotine) data from individual subjects. The positive diagonal line represents “no difference” and values falling below the diagonal line indicate improved post-treatment performance. Fig 2.4B uses similar scatter plots to compare nicotine vs. placebo data represented by post - pre

differences in the same subjects. Values clustered along the positive diagonal line indicate "no difference" and values falling below the diagonal line indicate lower tone-in-noise thresholds with nicotine than those with placebo. In general, we interpreted tone-in-noise thresholds below the diagonal line as improved performance attributed to increased central gain. Results show that tone-in-noise detection remained constant per frequency and level with both treatments. Tone-in-noise thresholds averaged ~30 dB SPL with ±5 dB spread per condition, and placebo and nicotine data largely overlapped (Fig 2.4A). Post – pre differences appeared to fall around 0 dB along the diagonal line indicating nicotine failed to significantly change detection thresholds in any tone-in-noise condition (Fig 2.4B, all $p > 0.17$).

We analyzed the variability of tone-in-noise thresholds by calculating the standard deviation across trials producing the last 6 reversals contributing to the average tone-in-noise threshold. For each tone-in-noise condition, we compared pre vs. post variability and nicotine vs. placebo variability and found no significant differences (data not shown, all $p > 0.04$).

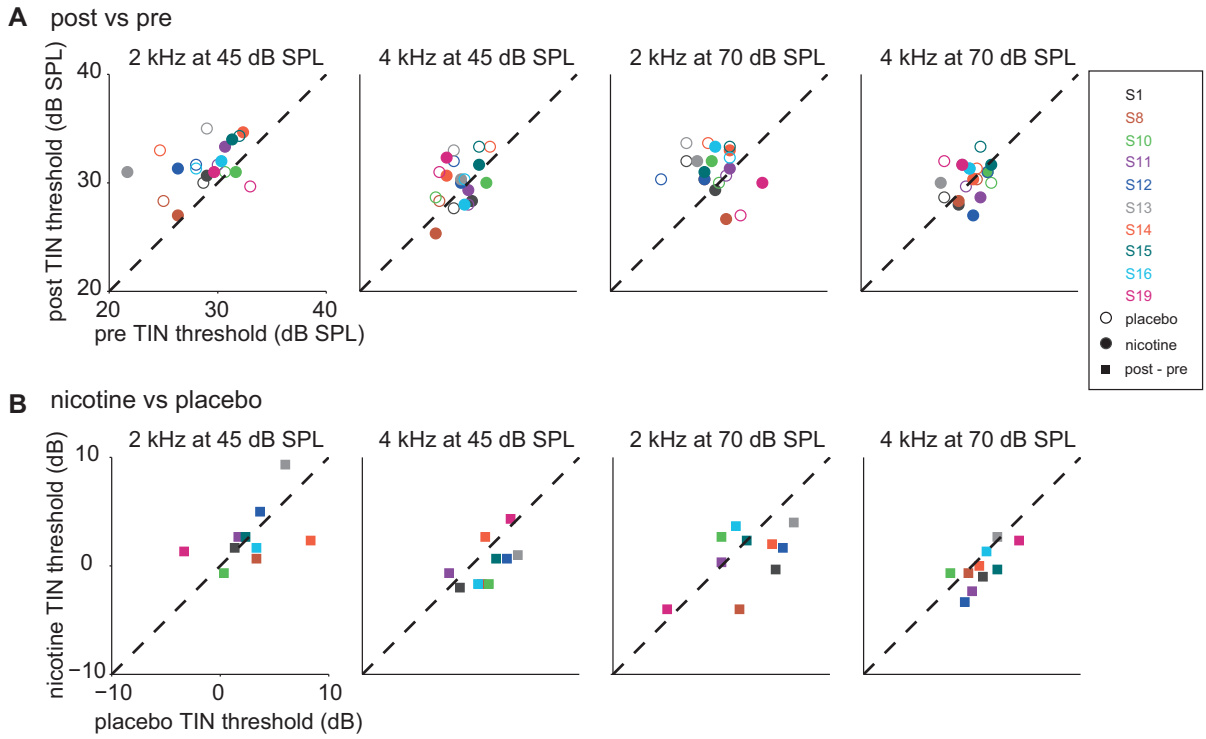

Figure 2.4 Tone-in-noise detection with placebo and nicotine.
Scatter plots show tone-in-noise (TIN) thresholds. Four conditions were tested (left to right columns): 2 and 4 kHz tone presented, respectively, at 45 dB SPL and 70 dB SL (~10 and ~40 dB sensation level, respectively) in 50 dB SPL noise as a measure of central gain. Different colors denote different subjects (n = 10). The positive diagonal line represents "no difference". **A** Post vs. pre TIN thresholds. Open and closed circles denote placebo and nicotine data, respectively. **B** Nicotine vs. placebo TIN thresholds. Squares denote post - pre differences.

Experiment II: Temporal gap detection

Fig 2.5 displays, in a similar scatter plot format as Fig 2.4, gap detection (gap threshold) in 11 subjects. Post gap threshold plotted as a function of pre gap threshold were compared on a logarithmic scale to compress the variance across BC and WC gap conditions (Fig 2.5A). The positive diagonal line represents "no difference" between pre and post gap thresholds. Values falling above or below the diagonal line indicate relatively lower pre or post gap threshold, respectively. Fig 2.5B shows nicotine gap threshold vs.

placebo gap threshold with values representing post - pre differences. As described previously, values clustered along the diagonal line indicate "no difference" and values falling below the diagonal line indicate relatively lower nicotine gap thresholds. In general, we interpreted gap thresholds below the diagonal line as increased detectability of shorter gaps attributed to increased temporal acuity. No differences existed between post and pre gap thresholds (Fig 2.5A). Individual WC gap thresholds clustered more tightly along the diagonal line compared to BC gap thresholds, which could reflect differences in task difficulty (e.g., larger spread indicates increased difficulty and task demand). Nicotine failed

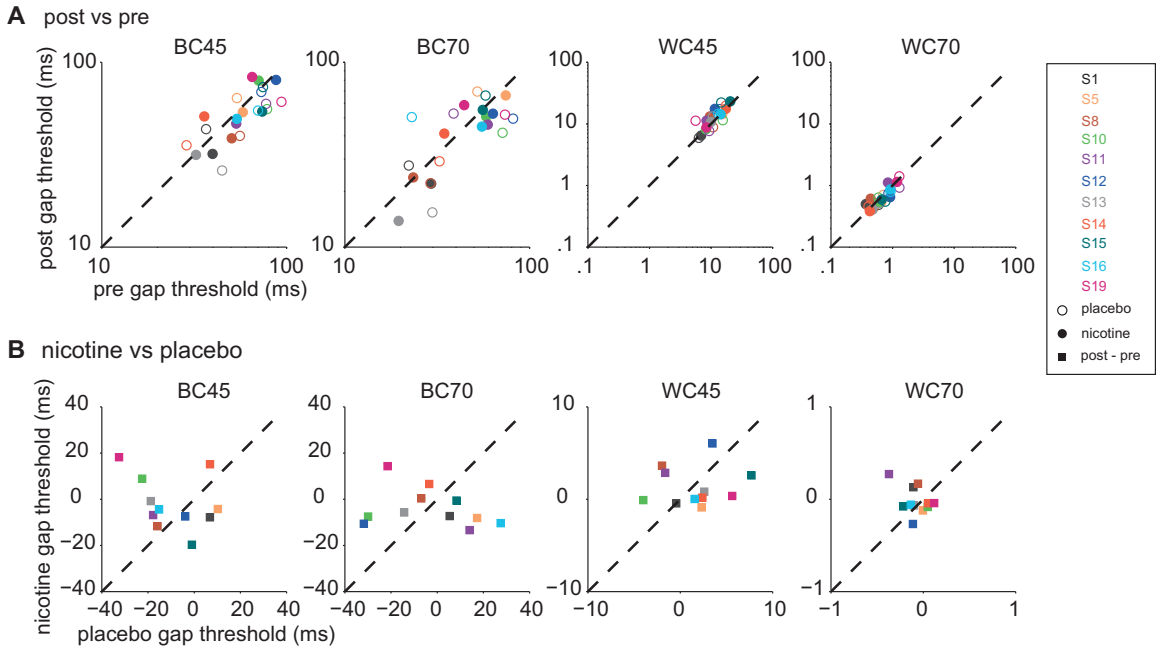

Figure 2.5 Temporal gap detection with placebo and nicotine.
Scatter plots show gap detection thresholds. Four conditions were tested (left to right columns): BC45, BC70, WC45, and WC70 as a measure of temporal acuity. Different colors denote different subjects (n = 11). The positive diagonal line represents "no difference". **A** Post vs. pre gap thresholds. Open and closed circles denote placebo and nicotine data, respectively. **B** Nicotine vs. placebo gap thresholds. Squares denote post - pre differences. BC45/ BC70: between-channel gap 4:2 kHz presented at 45 or 70 dB SPL; WC45/WC70: within-channel gap 2:2 kHz presented at 45 or 70 dB SPL.

to significantly change WC and BC gap thresholds at either sound level (Fig 2.5B, all p > 0.08).

Fig 2.6 compares more directly BC and WC conditions. As previously shown in Fig 2.5A, pre and post gap thresholds did not differ significantly therefore values were grouped per treatment. There were no significant drug effects on gap thresholds (red lines denote group means; all p > 0.01). Independent of level and treatment, BC gap thresholds exceeded WC gap thresholds. Interestingly, increasing level facilitated WC gap thresholds by an order of magnitude, however, BC gap thresholds changed by a relatively smaller amount (Fig 2.6). Considering how increased stimulus level did not improve BC gap detection as WC gap detection may suggest a critical locus for processing gaps between different frequencies outside the auditory system.

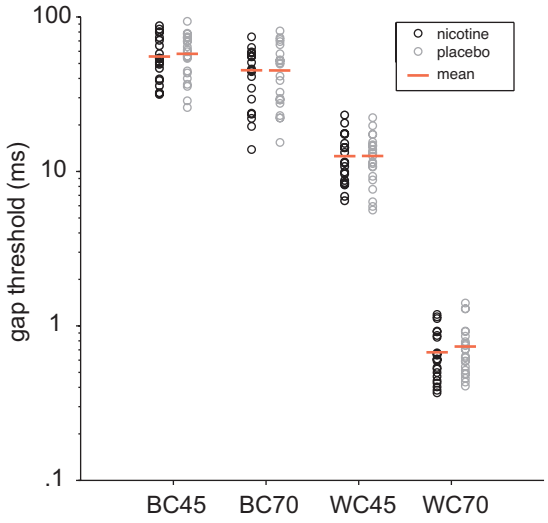

Figure 2.6 Summary of temporal gap detection. Individual gap thresholds with nicotine (black circles) and placebo (gray circles) are shown. Pre and post measurements are grouped per treatment. Red lines denote group means. Lower gap threshold signifying increased temporal acuity.

We analyzed the variability of gap thresholds by calculating the standard deviation across trials producing the last 6 reversals in the adaptive step. Fig 2.7A shows the post vs.

pre gap threshold variability. Fig 2.7B shows the nicotine vs. placebo gap threshold variability represented by post – pre differences. Six of 11 subjects had decreases in gap threshold variability post-nicotine (Fig 2.7A, BC45: S8, S10, S11, S12, S15, S16). Nicotine gap threshold variability just reached a significant difference in the BC45 condition (Fig 2.7B, p = 0.01), the most difficult gap condition. Increased task difficulty may relate to larger spread of gap threshold variability for BC gaps than for WC gaps (Fig 2.7A).

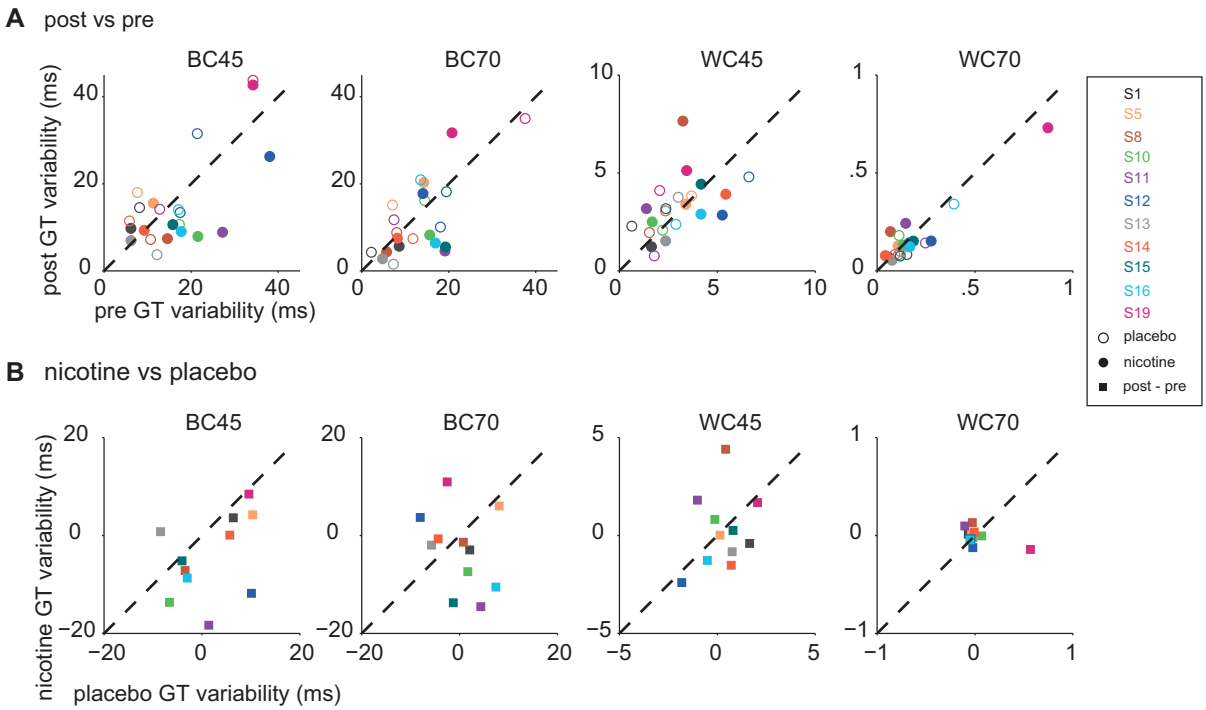

Figure 2.7 Variability in gap detection thresholds with placebo and nicotine.
Scatter plots show variability in gap thresholds (GT) calculated from the standard deviation across trials producing the last 6 reversals. Four conditions were tested (left to right columns): BC45, BC70, WC45, and WC70. Different colors denote different subjects (n = 11). The positive diagonal line represents "no difference". **A** Post vs. pre gap threshold variability. Open and closed circles denote placebo and nicotine data, respectively. **B** Nicotine vs. placebo gap threshold variability. Squares denote post - pre differences. There was a significant nicotine effect in the BC45 condition (p = 0.01). BC45/ BC70: between-channel gap 4:2 kHz presented at 45 or 70 dB SPL; WC45/WC70: within-channel gap 2:2 kHz presented at 45 or 70 dB SPL.

Experiment III: Spectral ripple discrimination

Fig 2.8 shows frequency selectivity measured in number of ripples per octave in 9 subjects using a similar format as Fig 2.4. A higher number of ripples per octave signifies better frequency resolution. Fig 2.8A shows post vs. pre ripple discrimination. Fig 2.8B shows nicotine vs. placebo ripple discrimination. On average, subjects could discriminate ~10 ± 2 ripples per octave. Nicotine failed to significantly change ripple discrimination (Fig 2.8B, $p = 0.35$).

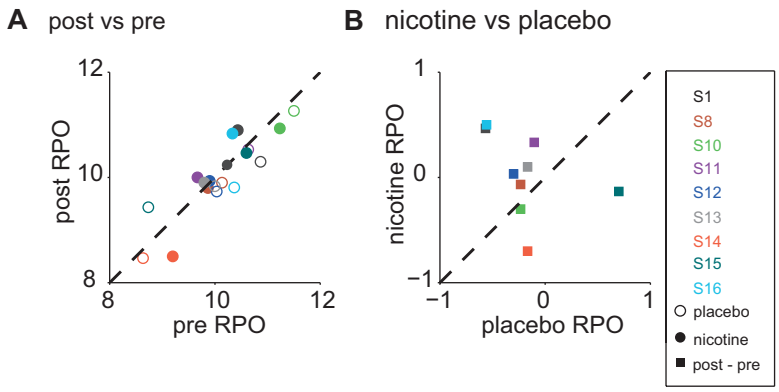

Figure 2.8 Spectral ripple discrimination with placebo and nicotine.
Scatter plots show frequency resolution in terms of ripples per octave (RPO). A higher number of RPO signifies increased frequency resolution. Different colors denote different subjects (n = 9). The positive diagonal line represents "no difference". **A** Post vs. pre ripple discrimination. Open and closed circles denote placebo and nicotine data, respectively. **B** Nicotine vs. placebo ripple discrimination. Squares denote post - pre differences.

Experiment IV: Attention in listening

We evaluated the speed of auditory information processing before examining specific attention effects of task relevant cues. In the Control condition, we measured mean reaction times for simply detecting the second tone without using frequency and location cues in 12 subjects (Fig 2.9; similar conventions as Fig 2.4). As a group, subjects responded

on average 300 ± 100 ms upon hearing the second tone independent of treatment. Largely overlapping reaction times indicated no post vs. pre differences (Fig 2.9A). Fig 2.9B shows nicotine vs. placebo reaction times as post - pre differences. Control reaction times appeared unaffected by nicotine (Fig 2.9B, $p > 0.63$).

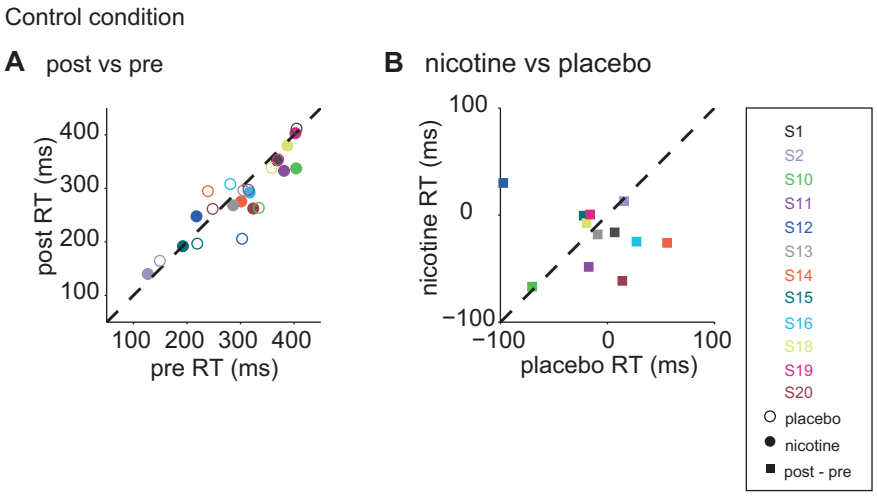

Figure 2.9 Reaction times in control condition with placebo and nicotine.
Scatter plots show the mean reaction times (RT) for responding to the second tone. Different colors denote different subjects (n = 12). The positive diagonal line represents "no difference". **A** Post vs. pre reaction times. Open and closed circles denote placebo and nicotine data, respectively. **B** Nicotine vs. placebo reaction times. Squares denote post - pre differences.

Reaction times given a task-relevant cue were the first attention measures we investigated. Fig 2.10 shows mean reaction times on correct trials in the FL (Fig 2.10A-B) and LF (Fig 2.10C-D) conditions in 12 subjects. The two tones were either the same (S) or different (D) in frequency (F) and/or location (L). We calculated baseline reaction time using the trials on which the two tones were the same in both frequency and location (SLSF). We also calculated reaction times for three other measures (DLSF, SLDF, DLDF). Fig 2.10A, C show individual post vs. pre reaction times. Fig 2.10B, D show individual nicotine

vs. placebo reaction times represented by post - pre differences. Pre and post reaction times appeared to largely overlap between treatments, ranging from ~400 ms - 900 ms in both FL and LF conditions (Fig 2.10A, C). Overall, nicotine had no main effect on reaction times in the FL condition (Fig 2.10B, all $p > 0.16$) or in the LF condition (Fig 2.10D, all $p > 0.03$). In general reaction times were shorter in the LF condition than in the FL condition. Both conditions produced group variance as large as ±100 ms around the means ranging from 500 – 600 ms. The mean reaction times for congruent measures (SLSF, DLDF) appeared shorter than for incongruent measures (DLSF, SLDF). Repeated reaction time measurements would have provided an estimate of intra-subject variance to better differentiate spread of reaction times per measure.

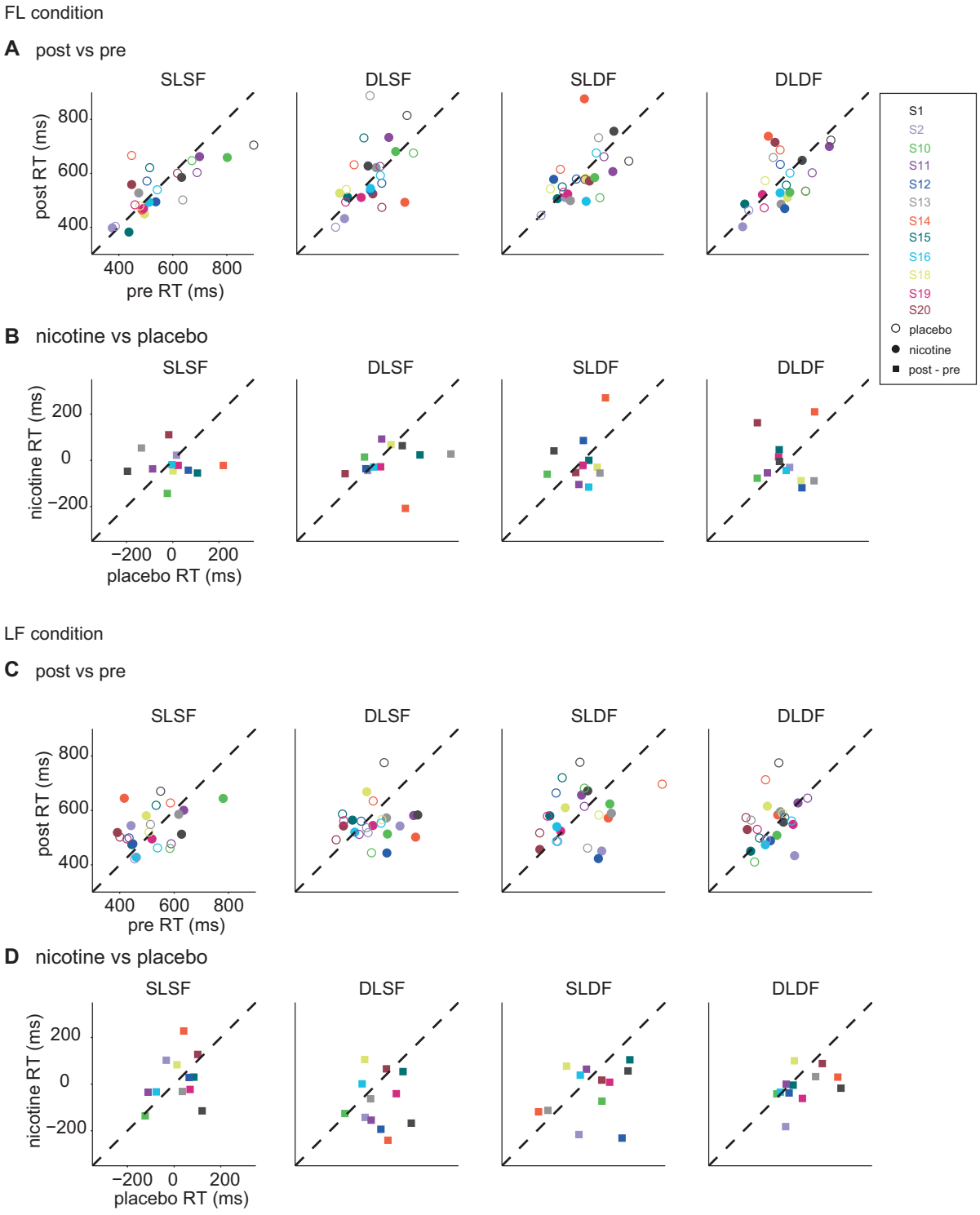

Figure 2.10 Reaction times in FL and LF conditions with placebo and nicotine.
Scatter plots show the mean reaction times (RT) on correct trials. The two tones were either the same (S) or different (D) in frequency (F) and/or location (L) cues. Averaged SLSF trials produced the baseline reaction time. Different colors denote different subjects (n = 12). The positive diagonal line represents "no difference". **A-B** FL condition. F served as the relevant cue and L served as the irrelevant cue. **C-D** LF condition. L served

as the relevant cue and F served as the irrelevant cue. Post vs. pre reaction times and nicotine vs. placebo reaction times are shown for both conditions. Open and closed circles denote placebo and nicotine data, respectively. Squares denote post - pre differences.

Our prediction that nicotine increases accuracy motivated investigation of error rates to examine the tradeoff between reaction time and accuracy. Fig 2.11 uses a similar scatter plot format as Fig 2.10 to show error rates in the 12 subjects previously mentioned. A random jitter of ~ 0.03 was added to error rates to better separate overlapping values. Error rates were lower in the LF than in the FL condition, consistent with shorter reaction times in the LF condition. Generally low mean error rates could reflect low attention demand in the task. Overall, the group performed with error rates below 0.1 (no mistakes at best) independent of treatment (Fig 2.11A, C). None of the four measures showed significant difference in error rate with treatment (Fig 2.11B, all $p > 0.52$; Fig 2.11D, all $p > 0.39$).

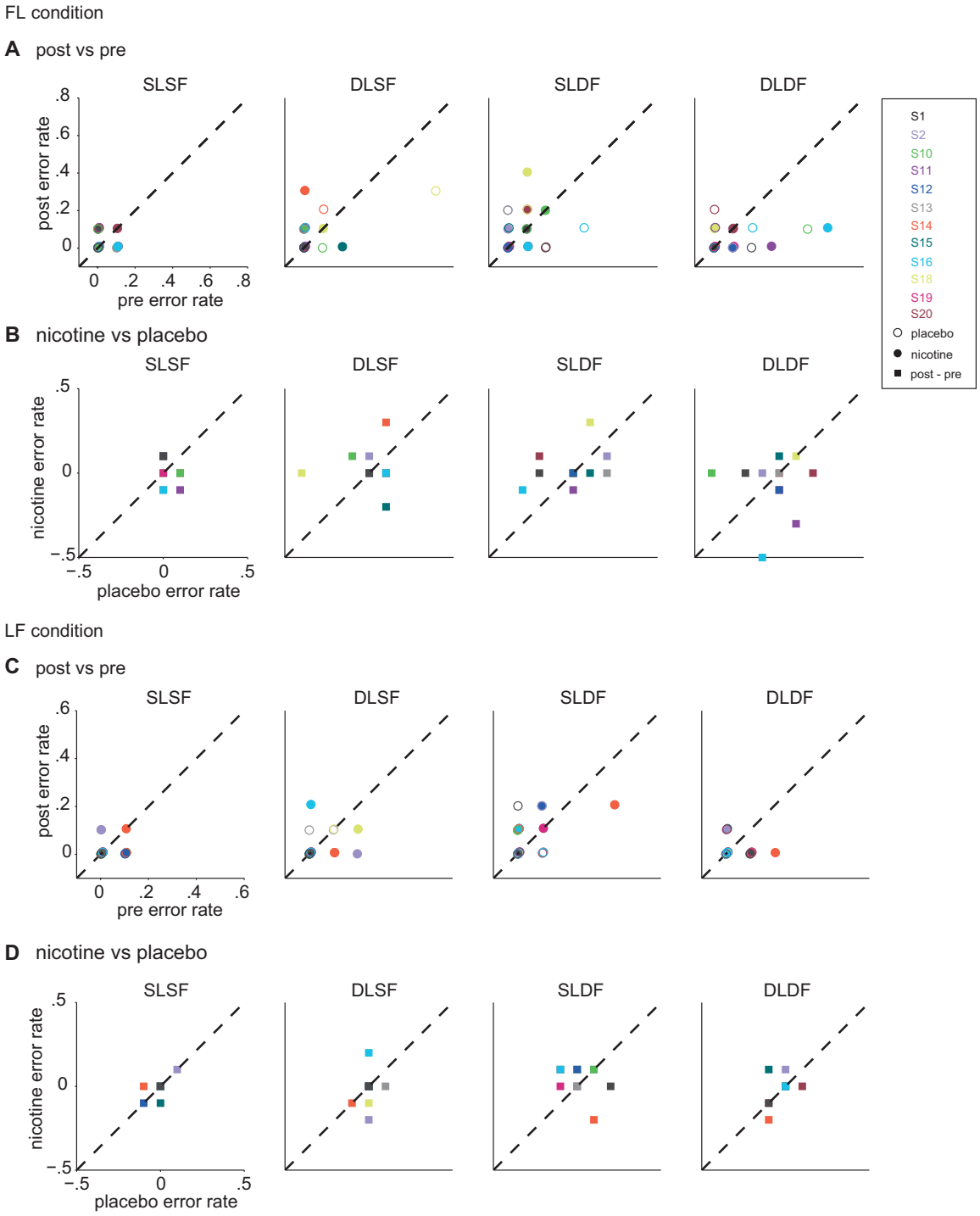

Figure 2.11 Error rates in FL and LF conditions with placebo and nicotine.
Scatter plots show error rates. The two tones were either the same (S) or different (D) in frequency (F) and/or location (L) cues. Different colors denote different subjects (n = 12). The positive diagonal line represents "no difference". **A-B** FL condition. F served as the relevant cue and L served as the irrelevant cue. **C-D** LF condition. L served as the relevant cue and F served as the irrelevant cue. Post vs. pre error rates and nicotine vs. placebo error rates are shown for both conditions. Open and closed circles denote placebo and

nicotine data, respectively. Squares denote post - pre differences. A random jitter of ~ 0.03 was added to error rates to better separate overlapping values.

We took a closer look at attention effects by grouping trials in which the two cues were congruent (SLSF, DLDF) or incongruent (DLSF, SLDF). Involuntary orientation refers to orienting attention to the task-irrelevant cue, e.g., in the FL condition, we measured involuntary orientating to location as the reaction time difference between different- and same- location trials (different- and same frequency trials in the LF condition). Conflict resolution can measure executive control in resolving cues containing conflicting information as the reaction time difference between incongruent and congruent trials. We expected nicotine, as a stimulus filter, to decrease susceptibility to distractors (involuntary orientation) and increase filtering of relevant cues while ignoring irrelevant cues (conflict resolution). In other words, nicotine enhances neural responses to relevant stimuli while suppressing neural responses to irrelevant stimuli. Fig 2.12 shows the derivative reaction times for involuntary orientation and conflict resolution in the FL (Fig 2.12A-D) and LF (Fig 2.12E-H) conditions. Fig 2.12A, E show post vs. pre reaction times for involuntary orienting. Fig 2.12B, F show corresponding nicotine vs. placebo reaction times. Fig 2.12C, G show post vs. pre reaction times for resolving conflicts. Fig 2.12D, H show corresponding nicotine vs. placebo reaction times. Nicotine produced no significant change in involuntary orientation regardless of attending to frequency (p = 0.76; Fig 2.12B) or location (p = 0.40; Fig 2.12F). Likewise, we found no significant difference in conflict resolution (FL: p = 0.53; Fig 2.12D; LF: p = 0.15; Fig 2.12H).

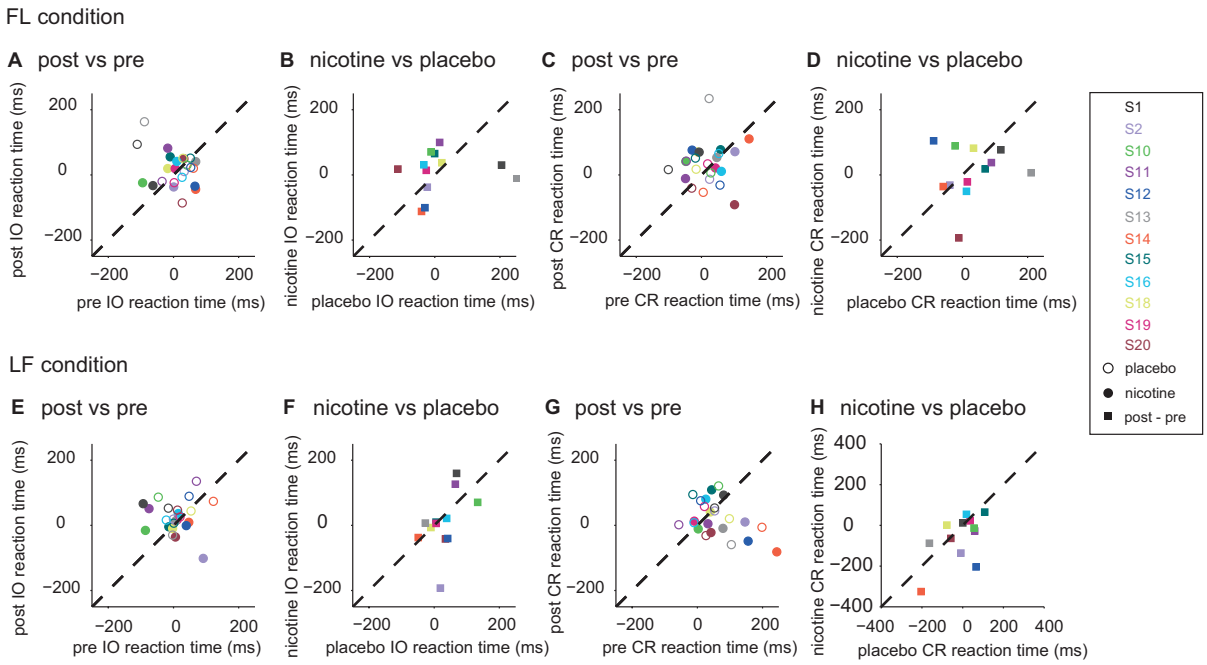

Figure 2.12 Derivative reaction times in FL and LF conditions with placebo and nicotine.
Scatter plots show derivative reaction times for involuntary orientation (IO) and conflict resolution (CR). Involuntary orientation is the difference between different and same distractor trials (e.g., in the FL condition (**A**-**D**), IO= different location – same location trials; in the LF condition (**E**-**H**), IO= different frequency – same frequency trials). Conflict resolution is the difference between incongruent and congruent trials [(same in one cue and different in the other) – (same or different in both cues)]. Different colors denote different subjects (n = 12). The positive diagonal line represents "no difference". Open and closed circles denote placebo and nicotine data, respectively. Squares denote post - pre differences. **A**, **E** Post vs. pre IO reaction times. **B**, **F** Nicotine vs. placebo IO reaction times. **C**, **G** Post vs. pre CR reaction times. **D**, **H** Nicotine vs. placebo CR reaction times.

We performed similar analyses of derivative error rates to quantify involuntary orientation and conflict resolution. Fig 2.13 uses a similar format as Fig 2.12 to show derivative error rates. As before, a random jitter of ~ 0.03 was added to error rates to better separate overlapping values. Fig 2.13A, E show post vs. pre error rates for involuntary orienting. Fig 2.13B, F show corresponding nicotine vs. placebo error rates. Fig 2.12C, G show post vs. pre error rates for resolving conflicts. Fig 2.12D, H show corresponding nicotine vs. placebo error rates. In summary, we found no significant drug

effects in involuntary orientation when attending to frequency (Fig 2.13B, p = 0.75) or location (Fig 2.13F, p = 0.47). Conflict resolution, similarly, did not reach significance level with treatment in the FL (Fig 2.13D, p = 0.35) or LF condition (Fig 2.13H, p = 1.0).

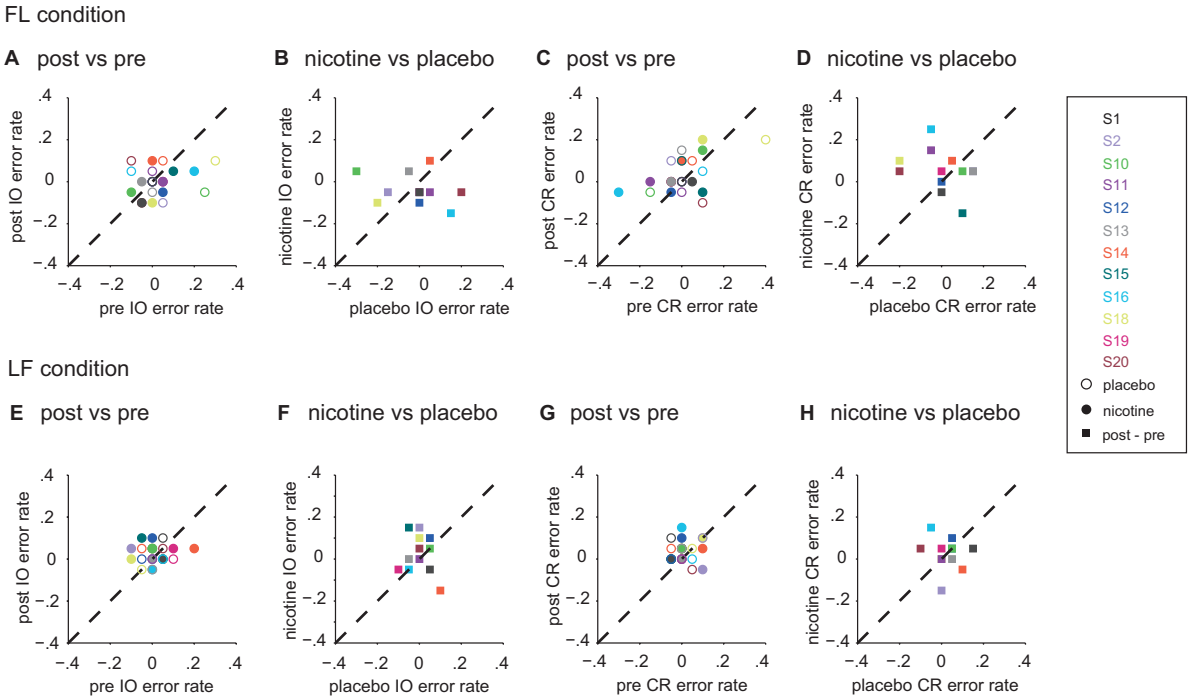

Figure 2.13 Derivative error rates in FL and LF conditions with nicotine and placebo.
Scatter plots show derivative error rates for involuntary orientation (IO) and conflict resolution (CR). Involuntary orientation is the difference between different and same distractor trials (e.g., in the FL condition (**A**-**D**), IO= different location – same location trials; in the LF condition (**E**-**H**), IO= different frequency – same frequency trials). Conflict resolution is the difference between incongruent and congruent trials [(same in one cue and different in the other) – (same or different in both cues)]. Different colors denote different subjects (n = 12). The positive diagonal line represents "no difference". Open and closed circles denote placebo and nicotine data, respectively. Squares denote post - pre differences. **A**, **E** Post vs. pre IO error rates. **B**, **F** Nicotine vs. placebo IO error rates. **C**, **G** Post vs. pre CR error rates. **D**, **H** Nicotine vs. placebo CR error rates. A random jitter of ~ 0.03 was added to error rates to better separate overlapping values.

Correlation analyses of attention effects on auditory processing

We performed a series of correlation analyses between attention effects (reaction times) and central gain (tone-in-noise), temporal acuity (gaps), and frequency selectivity (ripples) measures, respectively to study the contribution of attention on sensory processing. We expected conflict resolution measures to predict performance of auditory tests requiring executive control in distinguishing competing information, i.e., gap detection between frequencies. WC45 gap thresholds positively correlated with control reaction times independent of treatment. The linear regression accounted for ~61% (p = 0.01) and ~56% (p = 0.02) of the variance in pre- and post-placebo data, respectively, (Fig 2.14A) and for ~62% (p = 0.01) and ~80% (p = 0.001) of the variance in pre- and post-nicotine data, respectively (Fig 2.14B). These correlations could reflect matched attentional demand in detecting the second tone and in detecting a gap within frequency at the low sound level. We found lack of correlations between reaction times (control, involuntary orientation, conflict resolution) and central gain and frequency selectivity measures.

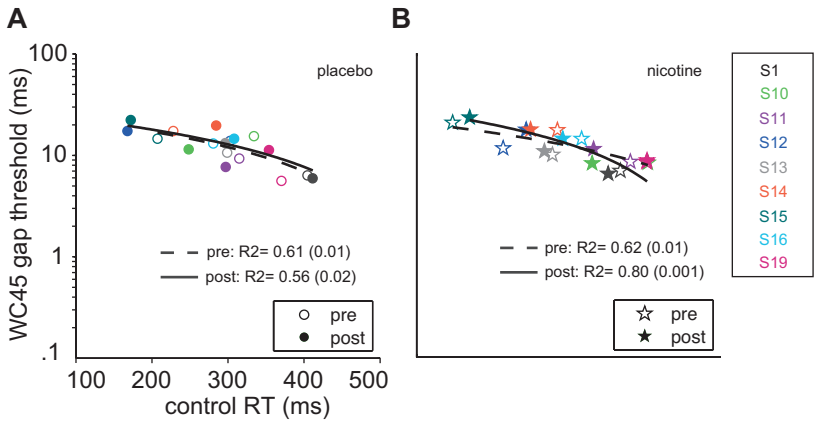

Figure 2.14 Correlation analysis of Control reaction time and within-channel gap detection.
Scatter plots show WC45 gap thresholds as a function of control reaction times (RT). Different colors denote different subjects (n = 9). Circles and stars denote placebo and nicotine, respectively. Open and close symbols

denote pre- and post- treatment, respectively. Dashed and solid lines show a significant linear regression in pre- and post- treatment measurements. **A** Pre- and post-placebo. **B** Pre- and post-nicotine. WC45: within-channel gap 2:2 kHz presented at 45 dB SPL

We also performed several correlation analyses comparing auditory processing tasks and found lack of correlations among measures of tone-in-noise detection, gap detection, and ripple discrimination measures. Fig 2.15 shows one example of a lack of correlation between WC and BC gap detection independent of sound level and treatment.

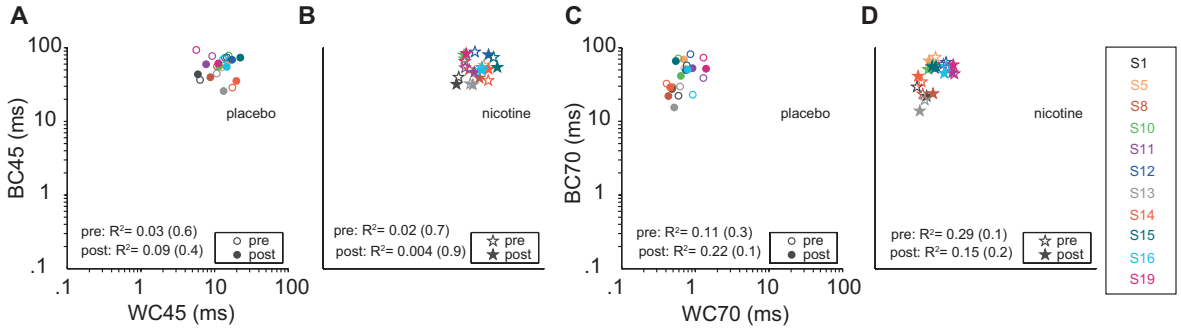

Figure 2.15 Correlation analyses of within- and between- channel gap detection.
Scatter plots compare BC to WC gap conditions. Different colors denote different subjects (n = 12). Circles denote placebo and stars denote nicotine treatment. Open and close symbols denote pre- and post-treatment, respectively. **A**, **C** Pre- and post-placebo **B**, **D** Pre- and post-nicotine. BC45/BC70: between-channel gap 4:2 kHz presented at 45 or 70 dB SPL; WC45/WC70: within-channel gap 2:2 kHz presented at 45 or 70 dB SPL.

DISCUSSION

The present study tested the hypotheses that nicotine increases auditory processing in terms of (1) central gain, (2) temporal acuity, (3) frequency resolution, and selective attention processing of (4) frequency and location cues. Our main results do not support the hypotheses. Oral nicotine has no significant main effects on central gain (tone-in-noise detection), temporal acuity (gap detection), or frequency resolution (ripple

discrimination). A secondary finding, however, seems to support our prediction that nicotine induces a change (albeit small) in auditory perception considering the robust nicotinic changes in auditory physiology (Intskirveli & Metherate, 2012; Kawai et al., 2007). Variability in gap processing between frequencies at low sound level (BC45) just reached a significant decrease with nicotine. Reaction times, error rates, and derived attention measures (involuntary orientation and conflict resolution) failed to change with nicotine. Furthermore, reaction times (Fig 2.10) and derived reaction times (Fig 2.12), respectively, do not correlate with central gain or frequency selectivity suggesting separate neural mechanisms govern selective attention and auditory processes. Control reaction time predicts gap processing within frequency at low sound level (WC45) independent of treatment. The lack of correlation between gap detection conditions suggests separate neural mechanisms mediate within- and between-channel gap processing (Phillips et al., 1997; Phillips, 1999).

Nicotinic decrease in gap threshold variability could reflect improved temporal summation

We think a significant change in variability of gap thresholds could reflect temporal summation. Kawai et al., 2007 show that systemic nicotine decreases the latency and latency variability of thalamocortical axon spikes. Evidence *in vitro* and *in vivo* show nicotine improves temporal summation for thalamocortical afferents converging onto the same neuron. Decreased latency variability in a population of afferents could increase spike summation and thereby amplitude of responses in the cortex. This presumed increase in central gain for gap signals may corroborate resolvability of temporal fluctuations in speech amidst competing background noise. We noticed nicotine effects in the more

difficult BC condition (BC45) in which dissimilar marker frequencies and low level signals can increase task demand and attention load relative to WC conditions. Larger threshold variability and significantly longer thresholds correspond to greater task difficulty in BC conditions relative to WC conditions (Fig 2.7). It may be that the nicotine effects on early attentional filtering become more evident under very demanding task conditions (Baschnagel & Hawk, 2008).

Task demand and individual baseline attentional processing limit nicotine effects

The lack of treatment effects on central gain (tone-in-noise threshold), temporal acuity (gap threshold), frequency resolution (ripples per octave) and auditory attention (reaction times and error rates) could result from healthy, normal-hearing subjects reaching optimal performance (Ernst et al., 2001; Smucny et al., 2015). Low task demand and high individual baseline attentional processing can explain optimal performance and lack of nicotinic effects. The attention allocation model asserts nicotine may act preferentially as a stimulus filter or stimulus enhancer dependent on task conditions (Kassel, 1997). Hence, the drug could increase or decrease recruitment of attention-associated brain areas as a function of task demands (Smucny et al., 2015). We tested different stimulus parameters and tasks in attempts to vary difficulty level/ task demand based on reports of nicotine improving attentional capacity and early attentional filtering in challenging task conditions (Baschnagel & Hawk, 2008; Knott et al., 2014a; Knott et al., 2014b). Low error rates suggest the TAIL conditions (Fig 2.11) were easy, posing little challenge to perceptual capacity (one tone presented at a time) (Zhang et al., 2012). Zhang and colleagues (2012) report the recruitment of executive control depends on task demand

as the conflict resolution effect disappears in simple detection of the second tone (Control condition). These results fit the prediction that increased executive control in the more challenging tasks reflect greater attention demand in conditions with conflicting cues. A taxing task that pushes the limits of attentional resources, presumably, engages more attention-meditating brain areas and show nicotine improvements (e.g., Stroop; Poltavski & Petros, 2006; Provost & Woodward, 1991). Newhouse et al., 2012 propose that the benefits of nicotine on attention relate inversely to baseline attentional functioning with greatest improvement in individuals with very poor baseline (or placebo) attention and actual impairment of attention among individuals with maximal baseline performance. Prior studies support this idea and lead us to think that subjects with lower baseline attention processing may exhibit stronger drug effects (Baschnagel & Hawk, 2008; Ernst et al., 2001; Harkrider & Hedrick, 2005; Knott et al., 2014a; Knott et al., 2014b). We also observed similar mixed patterns of improved and impaired subject performance across all of our perceptual measures. Future investigation of nicotine effects on attention-mediated auditory processing in clinical populations with loss of nAChRs (e.g., Alzheimer's, Levin et al., 2006; Sarter et al., 2009) which may impair the ability to filter stimuli and thereby affect attention in listening (central auditory processing disorders, Hurst et al., 2013) could show drug effects.

Correlation analyses propose different neural mechanisms govern attention and audition

The lack of correlations between attention (reaction time, involuntary attention, and conflict resolution) and auditory processing measures (tone and frequency selectivity) support separate attention and auditory processing systems (Posner & Petersen, 1990).

The positive correlation between gap detection (WC45) and control reaction time (Fig 2.14) could reflect matched attentional demand within one perceptual channel when simply detecting the second tone and detecting a gap within frequency at the low sound level but not high level.

The lack of correlation between WC and BC gap detection suggests different time-stamping processes limited by the auditory periphery and central computations, respectively. The high, cognitive level explanation by Fitzgibbons et al., 1974 propose BC gap detection involves a time-consuming process of shifting attention from the perceptual channel activated by the leading marker to the perceptual channel activated by the trailing marker. The lower level explanation by Phillips et al., 1997 propose BC gap thresholds increase above WC gap thresholds because perceptual or attentive resources allocated to any one channel (e.g., that representing the leading marker) reduces the resources available for the time stamping of events in any other channel. Taken together, we think temporal gap processing must rely on a dis-continuity in ongoing auditory nerve firing activity within the attended frequency channel in the auditory periphery and a cross correlation of nerve fiber discharges in different frequency channels integrated centrally by auditory areas and modulated by attention-associated brain areas. For example, the anterior cingulate gyrus cortex might play a role in attentional processing through selecting and recruiting processing centers appropriate for *auditory* task execution not just word/color processing (Pardo et al., 1990).

Limitations and future directions

Some study limitations include time constraints associated with the treatment testing protocol that did not regularly permit repeating measurements e.g., tone-in-noise and gap detection, and TAIL. In particular, repeating the 3 fixed TAIL conditions 2-3 times per subject would produce a measure of within-subject variability (Zhang et al., 2012). Additional analyses of variability in reaction times to study nicotine effects on sustained attention might be of interest. Ernst et al., 2001 found 4-mg nicotine gum significantly decreases variability (standard deviation) of reaction time to correct responses on a working memory task. Similarly, we could calculate standard deviation across reaction times of the 40 trials included in mean reaction times and compare variability between placebo and nicotine.

Arguably, oral nicotine may have provided insufficient nicotine blood concentration to significantly change perception as reflected by mild, self-reported side effects. Rodents systemically received a nicotine dosage (Intskirveli & Metherate, 2012) 10x greater (1 mg/kg nicotine hydrogen titrate) than that administered orally in human subjects (6 mg nicotine; 0.11 mg/kg for average 54 kg human), which would cause toxicity in humans (Hukkanen et al., 2005). We administered the highest oral dosage proven safe to test in humans (Knott et al., 2014a; Knott et al., 2014b). Alternatively, we could use transdermal nicotine (patch) to administer a higher nicotine concentration over a longer time course (e.g., nonsmokers: 7 mg/ 24 h, smokers: 21 mg /24 h; Harkrider & Hedrick, 2005). In the present study we chose oral nicotine instead due its short time to maximum plasma nicotine concentration and to minimize the incidence of adverse side effects leading to subject attrition. In non-smokers, however, nicotine effects on electrophysiological

consonant-vowel discrimination in quiet increases with severity of self-reported symptoms (Harkrider & Hedrick, 2005). A pilot clinical trial administering 6 months of transdermal nicotine (beginning with 5 mg and titrated to 15 mg by day 21) improves cognitive test performance in attention, memory, and psychomotor speed in nonsmoking, mild cognitively impaired subjects with safe tolerability (Newhouse et al., 2012). Future studies may examine nicotine effects using (titrated) transdermal nicotine or oral nicotine in clinical populations with lower attentional processing to study effects on the central auditory system.

Author Contributions

Conceived and designed the experiments: CQP, RM, and FGZ. Performed the experiments: CQP, SEG. Analyzed the data: CQP. Designed the software used for psychophysical testing: CQP, TL. Recruited subjects: CQP, SEG.

CHAPTER 3

Disrupted Auditory Nerve Activity Limits Peripheral but not Central Temporal Acuity

ABSTRACT

Auditory neuropathy disrupts auditory nerve activity in the periphery but its effect on central processing is unknown. The detection of a gap in a sound can be used to measure integrity of temporal processing mechanisms in the auditory system. To investigate the relative contribution of auditory nerve activity to peripheral and central temporal processes, we measured just-noticeable-differences in gap i.e., gap threshold between same- or different-frequency stimuli in both individuals with auditory neuropathy or normal hearing. Auditory neuropathy produces significantly worse than normal same-frequency but normal different-frequency gap detection. This suggests two distinct processes: a peripheral, within-channel mechanism mediating same-frequency gap detection and a central between-channel mechanism mediating different-frequency gap detection. The fast, peripheral mechanism enables temporal acuity on the order of milliseconds and is likely limited by neural synchrony, the amount of total nerve activity or both, while the sluggish, central mechanism on the order of ~100 milliseconds is likely limited by switching time between channels.

INTRODUCTION

Auditory neuropathy encompasses hearing pathologies that disrupt synchronous firing activity or reduce the total activity in the auditory nerve but spare audibility and amplification in the cochlea. Adults with auditory neuropathy often exhibit impaired speech comprehension in spite of having preserved pure tone audibility, preserved cochlear function indicated by outer hair cell measures such as cochlear microphonics and otoacoustic emissions (OAEs), but abnormal or absent auditory brainstem responses (ABRs) reflecting activity of auditory nerve and auditory brainstem pathways (Zeng et al., 1999; Rance, 2005; Starr et al., 1996; Wynne et al., 2013). These features accompany dysfunction of either auditory nerves (Kovach et al., 1999; Shaia et al., 2005; Starr et al., 2003) or inner hair cell ribbon synapses between hair cells and eighth nerve dendrites (e.g., mutations in *OTOF,* (Reisinger et al., 2011; Rodriguez-Ballesteros et al., 2008). The benefits of a cochlear implant for improving speech comprehension vary depending on the site and severity of auditory dysfunction. Electrical stimulation provides a clear benefit to patients with *OTOF* ribbon synapse disorders (Rouillon et al., 2006), whereas benefits vary for neural forms of auditory neuropathy (Berlin et al., 2010).

Starr and colleagues originally proposed that dis-synchronous auditory nerve afferent discharges arising from disruptions of the auditory periphery at the cochlea and/or the eighth nerve account for profound hearing deficits (Starr et al., 1991; Starr et al., 1996). Single auditory nerve fibers originating from the apical or low frequency portions of the basilar membrane discharge to signals up to 3 kHz during only one phase of the stimulus waveform and then only to a restricted portion of that waveform (Rose & Brugge, 1967). Eighth nerve fibers originating from the basal portion of the cochlea sensitive to

high frequencies preserve temporal cues through the precise latency of discharge to the onset of transient stimuli (Kiang et al., 1965). A presynaptic disorder of the ribbon synapse (*OTOF* mutations) affects neurotransmitter release, which in turn could alter the temporal precision of auditory nerve fiber discharges. Alternatively, a postsynaptic disorder or neural form of auditory neuropathy associated with axonal loss and demyelination of the eighth nerve (*MPZ or FX* mutations) affects neural conduction and transmission which in turn could disrupt the precise timing of neural discharges (Spoendlin, 1974; Starr et al., 2003). In a normal auditory system, synchronous neural discharges preserve the relative timing of action potentials propagated through several synaptic stages, which encode auditory features for speech recognition. The detection of a silent gap in a sound reflects the integrity of temporal processing mechanisms. Specifically, subjects with auditory neuropathy perform worse in detecting a gap in noise than subjects with sensorineural hearing loss or normal hearing (NH) which accounts for impaired speech-in-noise perception (Zeng et al., 1999; Zeng et al., 2005). In addition to neural dis-synchrony, reduced afferent input to the brain could also affect gap detection. Examination in one auditory neuropathy patient with a *MPZ* mutation revealed significant loss of auditory ganglion cells and central and peripheral auditory nerve fibers within the cochlea (Starr et al., 2003). A mouse model of spiral ganglion cell loss via ouabain treatment lesioned >95% of cochlear afferent synapses while sparing hair cells. Following this profound afferent degeneration, compensatory plasticity at higher stages of the CNS could recover sound feature representations supported by spike rate codes, but not spike timing codes (Chambers et al., 2016).

Evidence of impaired temporal gap processing of broadband noise (Zeng et al., 1999) motivated our gap detection study using frequency-specific stimuli to differentiate peripheral from central contributions to temporal processing. Poor gap detection at low sensation levels also suggests reduced peripheral input as a second mechanism for temporal deficits in auditory neuropathy. Proposed peripheral mechanisms limiting temporal acuity of the auditory system comes from records showing single auditory nerve fiber activity represent the gap stimulus in the time course of spike discharges. The shortest gaps clearly correspond with a dip or discontinuity in the ongoing discharge rate of auditory nerve fibers, which also approximate behavioral gap thresholds (Zhang et al., 1990). Temporal acuity may be limited by the slow decay of sensation during the gap, which prevents the silent interval from being detected (Plomp, 1964). Discontinuous firing in ongoing neural discharges may explain gap detection in identical stimuli but does not completely account for gap detection in dissimilar stimuli. The latter is limited, presumably, by centrally computed cross-correlation of activity between different neural populations and relative timing of activity representing the offset of the leading sound and the onset of the lagging sound following the gap. Under this framework, Phillips and colleagues designed stimuli consisting of identical components (markers) bounding a gap that stimulate overlapping population(s) of peripheral auditory neurons which activate one perceptual auditory channel (within-channel, WC) and spectrally dissimilar markers that stimulate non-overlapping populations of peripheral auditory neurons which activate different perceptual channels (between-channel, BC)(Phillips & Hall, 2000; Phillips & Smith, 2004; Phillips, 1999).

Here we evaluated gap detection in subjects with auditory neuropathy: two with ribbon synapse disorder, one with neural disorders, and one with unknown origin, and one subject with acoustic neuroma. Auditory neuropathy has been shown to disrupt auditory nerve activity in the periphery, but its effect on central processing is unknown. We hypothesized a peripheral pathology related to abnormal auditory nerve activities would affect gap detection within frequency and between frequencies, whereas a central pathology would affect only gap detection between frequencies. We tested gap detection between pairs of tones or noisebands centered on the same frequency (WC) or different frequencies (BC) to determine the relative contribution of the auditory nerve to peripheral vs. central temporal acuity.

METHODS

Ethical statement

The University of California Irvine Institutional Review Board approved all study protocols for testing human subjects. Each subject provided written informed consent before participating in the study and received compensation for testing.

Subjects

Four subjects pre-diagnosed with auditory neuropathy participated in the study. In order to follow the progression of disorders the auditory neuropathy subject code used in the present study refers to identical subjects with auditory neuropathy or acoustic neuroma who were tested in our previous publications (Dimitrijevic et al., 2011; Michalewski et al., 2009; Wynne et al., 2013; Zeng et al., 2005). Table 3.1 contains data

about site of disorder (synaptic or neural), demographics (age, sex), audibility (pure tone average across ears), ABRs (wave V), gap detection thresholds (gap in noise), gene mutation (if known), and special clinical features. Prior to the gap detection experiments, measurement of OAEs and ABRs confirmed auditory neuropathy diagnosis in one new subject (AN41) and tracked the longitudinal progression of auditory nerve disorders in previously tested subjects (data not shown) (Starr et al., 1998; Wynne et al., 2013). Cochlear microphonics appeared normal in all 4 auditory neuropathy subjects. Otoacoustic emissions were present in 3 of 5 subjects. ABR wave V latency appeared abnormal in 3 subjects and absent in 1 subject. Two subjects with auditory neuropathy (Subjects AN32, AN33) were siblings, have compound heterozygous mutations of *OTOF*, which cause 'deafness' at elevated body temperature (Marlin et al., 2010; Starr et al., 1998; Varga et al., 2006; Wang et al., 2010). Both audiometric thresholds and speech perception in quiet remain normal or mildly affected at normal body temperature. Mutations in *OTOF* affect release of neurotransmitters from highly specialized ribbon synapses of inner hair cells (Pangrsic et al., 2010; Roux et al., 2006). Subject AN13 expresses involvement of other cranial (optic, vestibular) and/or peripheral nerves of unknown etiology. Subject AN41 is a monozygotic twin with bilateral auditory neuropathy, presumably, resulting from perinatal conditions related to premature birth and hypoxia during delivery. One subject with hearing impairment resulting from an acoustic neuroma served as a control subject for low frequency hearing loss frequently occurring in auditory neuropathy (Zeng et al., 1999) and present in our 4 subjects with auditory neuropathy. The subject had a small (2.5mm) acoustic neuroma in the right ear treated by gamma knife radiation. She developed low frequency hearing loss after the procedure. Our previous tests revealed the subject had

absent OAEs and ABR Wave V in the right ear (Wynne et al., 2013). The right ear with the tumor also served as a control for proximal site of auditory nerve involvement associated potentially with abnormal temporal processing. The left ear with acoustic hearing had normal thresholds up to 2 kHz and gradually increasing hearing loss up to 70 dB HL at 4 kHz and no response up to 90 dB HL at 8 kHz.

Six subjects (age 18–44 years, male/ female: 3/6) with relatively normal audibility (≤20 dB HL (decibel Hearing Level) for pure tone audiometry between 0.125-12 kHz) participated as normal hearing controls in the experiments. Specifically, Subjects NH1, NH2, NH3, and NH4 (AN41's twin) served as age- and sex- matched controls for Subjects AN32, AN33, AN13, and AN41, respectively. Fig 3.1 shows the audiogram of pure tone thresholds averaged between ears (except AN13 who has a cochlear implant in the right ear) in auditory neuropathy subjects (colored solid lines) and thresholds measured in right ears in normal hearing subjects (colored dashed lines). In each auditory neuropathy subject, both ears were tested (except for AN13). A two-sample Kolmogorov-Smirnov test revealed no significant difference between ears (p = 0.45) therefore we grouped and averaged each subject's pure tone thresholds per frequency (Fig 3.1). In general, subjects in the auditory neuropathy group have moderate-to-severe hearing loss.

Subject #	Site of AN	Age	Sex	Ear tested	OAEs	ABR wave V latency	PTA (dB HL)	Gap (ms)	Gene	Special features
AN32[+]	Synapse	25	F	Left Right	Yes	Abnormal	35	12	OTOF	Temperature Sensitive
AN33[+]	Synapse	22	M	Left Right	Yes	Abnormal	39	11	OTOF	Temperature Sensitive
AN13[+]	Nerve	44	F	Left	Yes	Absent	84	8	?	Peripheral Neuropathy
AN41[++]	?	14	M	Left Right	No No	Absent Abnormal	58	DNT	?	Peripheral Neuropathy
Neuroma[+]		66	F	Right Left	No DNT	Absent DNT	39	115 DNT		Acoustic Neuroma
Total	5	14-66	3F 2M	9	3/5	3/5	51	36.5		

Table 3.1. Features of subjects with auditory neuropathy or acoustic neuroma.
Synapse = ribbon synapse disorder; Nerve = neural AN; OAEs = Otoacoustic Emissions; ABR: Auditory Brainstem Responses; PTA= Pure Tone Threshold Average across octave frequencies between 0.125 and 12.0 kHz across ears; Gap= Gap in Noise; DNT = did not test; ? = unknown; *OTOF* = Otoferlin; OAEs, ABRs, and gap thresholds reported in [+] Wynne et al., 2013. OAEs and ABRs tested by [++] Providence Hearing and Speech Clinic.

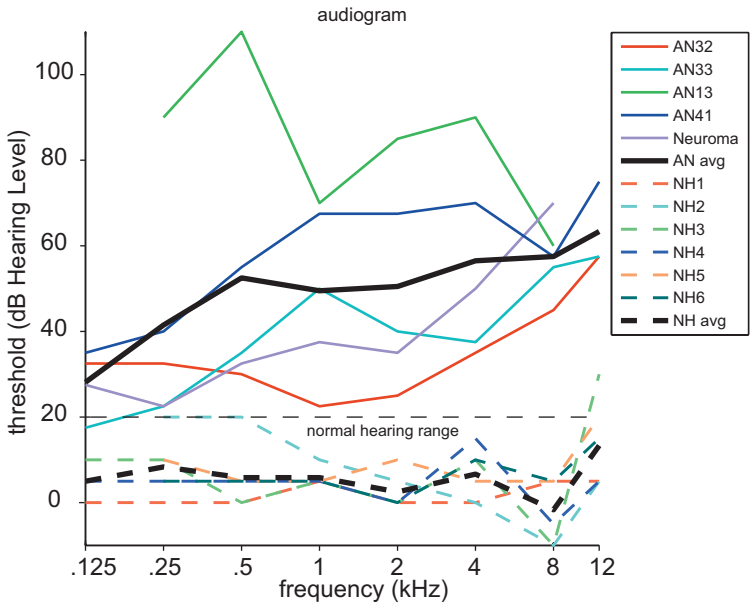

Figure 3.1 Audiogram.
Different color lines indicate different subjects. Dashed lines represent normal hearing (NH) subjects and solid lines indicate auditory neuropathy (AN) subjects. Pure tone thresholds were averaged between ears (except AN13 who has a cochlear implant in the right ear) in AN subjects and only thresholds measured in right ears in NH subjects. The thick, black, dashed and solid lines indicate the pure tone average for the NH and AN group, respectively. Thresholds that fall below the thin, dashed line indicate the normal hearing range for pure tones.

Stimuli

All acoustic stimuli were generated in MATLAB and presented monoaurally through calibrated circumaural headphones (HDA-200, Sennheiser electronic GmbH & Co. KG, Wedemark, Germany). Stimuli presented to a subject with auditory neuropathy or hearing impairment passed through a power amplifier (Crown Audio device D-75, two channel, 55W Power) and were adjusted to and presented at comfortable loudness level. Stimuli presented to a normal hearing subject were amplified via a sound card (Creative Labs E-MU 0404 USB input) before playing though the headphones.

A gap stimulus contained a leading and lagging marker bounding a silent gap at the center of a fixed stimulus interval. The amplitude envelope for the leading and lagging markers was shaped with 2.0-ms linear rise-fall times, including those defining the gap. To create a WC gap stimulus within a frequency channel both leading and lagging marker envelopes were multiplied by a .5, 2, or 4-kHz sinusoid (.5:.5; 2:2; 4:4 kHz). Conversely, to create a BC gap stimulus between different frequencies, the leading marker envelope was multiplied by a 4-kHz sinusoid and the lagging marker envelope was multiplied by a 2-kHz sinusoid (4:2 kHz). The summed envelopes produced a gap stimulus with two tonal markers. The total stimulus interval duration remained constant for both groups. A stimulus interval of 800 ms, longer than that use to test normal hearing subjects, was used to make the task easier for auditory neuropathy subjects. Adaptively changing gaps caused marker durations to range between 125-250 ms (normal controls) and 200-450 ms (auditory neuropathy). Initial gap duration was a short as 5-10 ms (WC) and as long as 250-450 ms (BC). All normal control subjects heard 500 ms stimulus intervals. We tested two control subjects using 800 ms stimuli and found gap threshold varied < ~1 ms

compared with 500 ms stimuli. A fixed stimulus interval removed duration cues potentially created as the gap duration changed. A 500-ms inter-stimulus interval separated each stimulus. The duration of each gap was specified by the time between the -6 dB point at the end of the leading marker envelope and the equivalent point at the beginning of the lagging marker envelope. The no gap stimulus contained a gap of 0 ms in which the -6 dB point of the leading marker envelope offset overlapped at the equivalent point of the lagging marker envelope onset to create a perceived, uninterrupted stimulus. Tone components were presented at 70 dB SPL (~40 dB sensation level) and summed with a 50 dB SPL pink noise component. As previously described, pink noise was designed to minimize spectral splatter produced by abrupt gating of the gap (5-octave pink noise centered on 2828 Hz with -3 dB/octave slope). Moderate level noise partially filling the temporal gap did not interfere with the subject's ability to detect the gap. A total of four stimulus conditions were tested: .5:.5, 2:2, 4:4; 4:2 kHz.

Gap in noise stimuli were tested for comparison with past gap detection in the same auditory neuropathy subjects. Narrowband noise was selected for the purpose of testing a between-channel condition. A temporal gap was placed in the center of 1-octave, bandpass noise 24 dB/oct (4^{th} order Butterworth), filtered uniform white noise. The leading and lagging markers were either centered on 2 kHz (WC) or 4 kHz and 2 kHz (BC), respectively. A 2 ms-ramp shaped the noise stimuli. The noise stimulus interval was 500 ms.

Stimuli were presented at 70 dB SPL to control subjects or between 65 to 105 dB SPL, corresponding to each auditory neuropathy subject's most comfortable loudness level (subjective rating of 6/10 on a standard 10-interval loudness scale). All stimulus presentation was at ~40 dB sensation level: dB re: the subject's threshold for that stimulus.

Procedure

All auditory testing took place in a double-walled sound-attenuating booth. Auditory neuropathy subjects rated loudness level for each stimulus as the experimenter adjusted the dB SPL until the perceived stimulus was at comfortable loudness level. These sound levels were used in the gap detection experiment. A 3-interval forced choice, adaptive procedure measured subjects' just-noticeable-differences in gap (gap threshold) between identical or different tone or noise bursts, respectively. Only subject AN32 and AN33 and control subject NH1 performed gap in noise detection. During each trial, the subject heard three stimuli visually marked by three intervals on a computer screen. One of the three intervals at random (3-alternative) contained the gap stimulus while the other two contained the no gap stimulus. The subject had to select the gap stimulus (forced-choice) and received visual feedback on the correct response. The initial difference between the gap and no gap stimulus was large so it was easy for the subject to distinguish which interval contained the gap. The gap duration reduced by a factor or 2 after two consecutive correct responses and increased by the same factor after one incorrect response (2-down, 1-up), corresponding to 70.7% percent correct response performance (Levitt, 1971). A reversal was recorded when the subject made an incorrect response from two or more consecutive correct responses or vice versa. Each threshold track persisted until reaching a total of 10 reversals and repeated at least 3 times per ear. The reported gap thresholds represent the averaged of the last 6 reversals. The stimulus conditions were tested in random order with training beginning with 2:2 kHz gap detection (presumably easier than 4:2 kHz gap detection).

Statistics and data analysis

We plotted gap thresholds on a logarithmic scale to reduce the unequal variance in BC and WC conditions. A two-sample Kolmogorov-Smirnov test comparing all tonal gap thresholds from AN listeners revealed no significant difference (p = 0.11) between left and right ears justifying subsequent analysis of data grouped across ears and for comparison with normal control right ears. We included the subject with the acoustic neuroma in the auditory neuropathy group considering similar pattern of thresholds with auditory neuropathy subjects.

We used a linear mixed model ANOVA with: (1) condition as the within-subject (4 levels: .5:.5, 2:2, 4:4; 4:2 kHz) and (2) group as the between-subject factor (2 levels: auditory neuropathy, normal hearing) for all comparisons as this model provides enough degrees of freedom to estimate the within-subject effect for the auditory neuropathy group. *Post-hoc* independent samples t-tests were used to test significant differences in conditions between groups. A significance level of $p < 0.05$ was used with Bonferroni adjustments.

We used a linear regression with a least-squares criterion to assess correlations between gap conditions, hearing loss, and stimulus level to address issues associated with hearing impairment potentially confounding gap detection performance.

All statistical analyses were performed in SPSS for Windows (version 11.0, 2002, Chicago, Illinois: SPSS Inc.) and MATLAB.

RESULTS

Fig 3.2 uses a boxplot to show WC and BC gap detection performance for tones in

subjects with auditory neuropathy (red boxes) and normal controls (black boxes). Comparing groups with all three WC conditions pooled, Fig 3.2A shows WC thresholds (median ± SD) from the NH group (0.9 ± 2.0 ms) and the AN group (5.0 ± 34.0 ms). Subjects with auditory neuropathy perceived within-frequency gaps approximately an order of magnitude longer than NH controls. Surprisingly, both groups detected similar range between frequency gaps of 44 ± 47 ms (control) and 45 ± 24 ms (auditory neuropathy). As expected from previous reports in normal hearing listeners, between frequency gap thresholds were longer than within-frequency gap detection by our control subjects (Phillips et al., 1997; Phillips & Hall, 2000; Phillips, 1999). We, however, unexpectedly also observed this pattern in subjects with auditory neuropathy.

Fig 3.2B provides a closer look at WC gap detection of low, mid, and high frequencies (.5, 2, and 4 kHz) compared with BC gap detection and individual variability. Different symbols represent different subjects with black and gray symbols denoting individual median thresholds and repeated threshold measurements. On a group level, an effect of increasing gap threshold with frequency emerged in the control data. Auditory neuropathy data also reflected this trend. Subjects with auditory neuropathy appeared to have larger WC gap thresholds variability compared to that in normal controls. Individual normal hearing variability appeared larger for gaps between frequencies than for gaps within frequency, consistent with previous reports (Phillips et al., 1997; Phillips, 1999). The opposite pattern occurred in individual auditory neuropathy variability with larger spread in WC gap thresholds than BC gap thresholds. The upper limit of the normal hearing range (spread in gray circles) provided a criterion for determining abnormal performance in that auditory neuropathy (AN) thresholds above normal hearing (NH) variability suggest

abnormal gap detection. Gap threshold range for the .5:.5 kHz condition were 0.1 – 1.0 ms (NH, median = 0.3 ms) and 0.2 - 169 ms (AN, median = 3.0); for the 2:2 kHz condition: 0.4 – 9.0 ms (NH, median = 0.8 ms) and 0.4 – 66 ms (AN, median = 4.0 ms); for the 4:4 kHz condition: 1.0 – 3.0 ms (NH, median = 1.7 ms) and 2.0 – 53 ms (AN, median = 13 ms); and for the 4:2 kHz condition: 15 – 164 ms (NH, median = 44 ms) and 21 – 84 ms (AN, median = 45 ms). Subject AN32 (red square) seemed to have normal WC gap detection of .5 kHz but abnormal detection of 2 and 4 kHz by about an order of magnitude. Specifically, gap threshold within frequency were higher in the left ear, which had slightly greater hearing loss (see upper gray squares in 2:2 and 4:4 kHz conditions). Subject AN13 (green inverted triangle) had elevated gap thresholds across all gap conditions. Subject AN41 (blue triangle) had elevated gap thresholds at all frequencies exceeding those in his normal hearing twin (Fig 3.2B, circles in 6th column of boxes). The subject with neuroma (purple diamond) seemed to have high thresholds for .5 and 4 kHz but within normal range thresholds for 2 kHz. Auditory neuropathy subjects appeared to have normal BC gap detection of 20-100 ms long. In another BC condition (4:5 kHz; data not shown), subject AN32 and the subject with acoustic neuroma had similar gap detection as one normal control subject (NH1), which alludes to normal BC gap processing in these subjects and may result in the same or different pattern with increased power.

A mixed linear model revealed a non-significant group x condition interaction effect ($F_{(3,8)} = 3.33$, $p = 0.08$) and group effect ($F_{(1,8)} = 3.0$, $p = 0.12$) and potentially significant condition effect ($F_{(3,8)} = 21.6$, $p < 0.05$). *Post-hoc* independent samples t-tests revealed that none of the four gap conditions condition comparing the AN to the NH group reached significance with Bonferroni corrections. The 4:4 kHz condition, however, was approaching

significance level (p = 0.0125) with adjustments ($t_{(3)}$=-3.22, p = 0.048). The present study is likely underpowered to observe effects of gap condition and group effects.

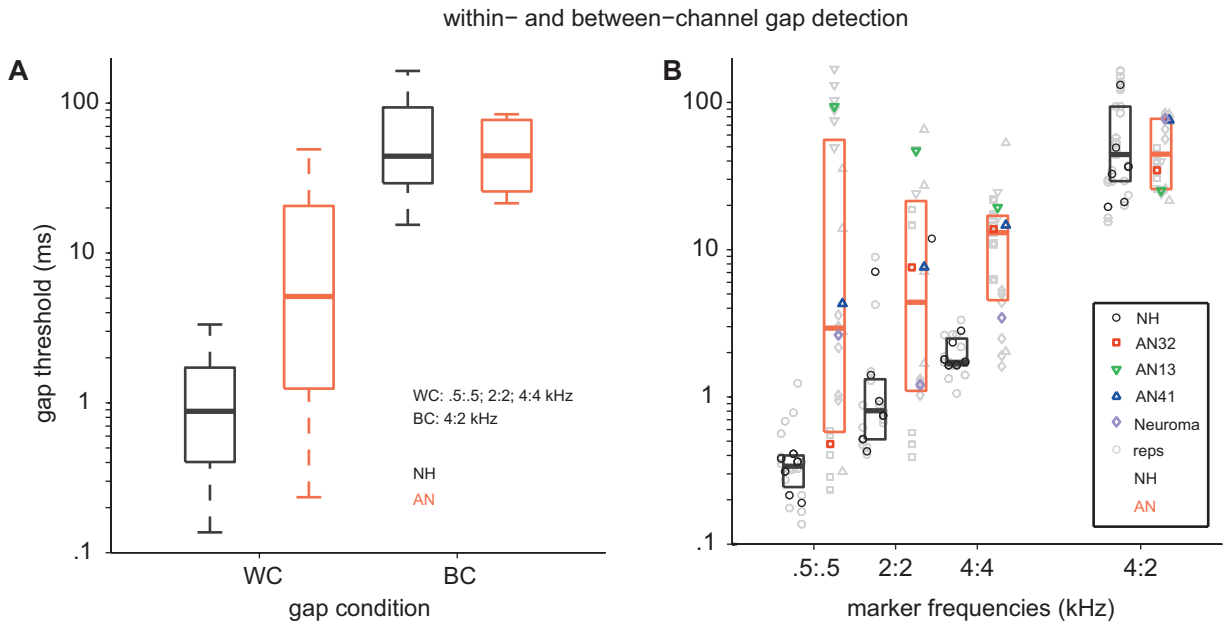

Figure 3.2 Tonal within- and between-channel gap detection in auditory neuropathy and normal hearing groups. Black and red boxes represent data from normal hearing (NH) and auditory neuropathy (AN) groups, respectively. The mid-line of the boxes indicate the group median and the lower and upper lines, respectively, indicate the 25th and 75th quartiles. Gap threshold represents duration of the detected gap when bounded by the same frequency or different frequencies. Within-channel (WC) conditions tested frequencies at .5:.5, 2:2, 4:4 kHz. Between-channel (BC) conditions tested frequencies at 4:2 kHz. Lower values indicate better gap detection performance. **A** Whisker lines indicate the range. **B** Different symbols and colors indicate different subjects. Data from Subject NH1-6 are shown as respective columns within each black box. Black symbols represent individual subject's median threshold and gray symbols represent individual repetitions of threshold measurements.

We tested gap detection in narrowband noise to follow-up on disorder progression in Subject AN32 and AN33 (data not shown; Starr et al., 1998; Wynne et al., 2013). One control subject (NH1) had gap thresholds (median ± SD) of 5.0 ± 0.7 ms (WC) and 20 ± 6.0 ms (BC). Subject AN32 had gap thresholds of 13 ± 4.0 ms (WC) and 30 ± 11 ms (BC). Subject AN33 had gap thresholds of 16 ± 7.0 ms (WC) and 25 ± 4.0 ms (BC). Within-channel

gap thresholds in both subjects with ribbon synapse disorder fell within the order of tens of ms which seemed slightly longer than previously measured gap thresholds in the same subjects (Table 3.1; Wynne et al., 2013) but consistent with the auditory neuropathy range for detecting gaps in broadband noise (Zeng et al., 1999; Zeng et al., 2005). As observed with testing tonal gaps in both groups, BC noise bursts differing in center frequency tended to produce longer thresholds compared with those produced by WC identical noise bursts. Comparing results obtained with tone and noise stimuli, we found WC thresholds for noise tended to exceed WC thresholds for tones, however, BC thresholds overlapped in range (compare gap in noise thresholds with 2:2 and 4:2 kHz tone conditions in Fig 3.2B).

The large differences between threshold for gaps within frequency and gaps between frequencies led us to perform correlation analyses to study relationships between proposed neural mechanisms. Fig 3.3A compares low frequency .5 kHz WC gap thresholds with high frequency 2 kHz and 4 kHz WC gap thresholds, respectively (open circles: NH; filled circles: AN). For the auditory neuropathy group, the positive correlation between high frequency thresholds and low frequency thresholds in the WC condition accounted for 80% of the variance (p = 0.02). The lack of correlation in control data arise likely from ceiling performance (R^2 = 0.32, p = 0.31). Fig 3.3B compares BC gap thresholds with WC gap thresholds. Different symbols denote different WC frequencies. Our results seem consistent with previous findings that within-channel 1 kHz and 4 kHz gap thresholds correlate but 1 kHz and 4 kHz within-channel gap thresholds each poorly predict 4-1 kHz between-channel gap thresholds in the same listeners (Phillips & Smith, 2004; Phillips, 1999). The lack of correlation in both groups suggests separate neural mechanisms mediating detection of gaps within frequency and between frequencies.

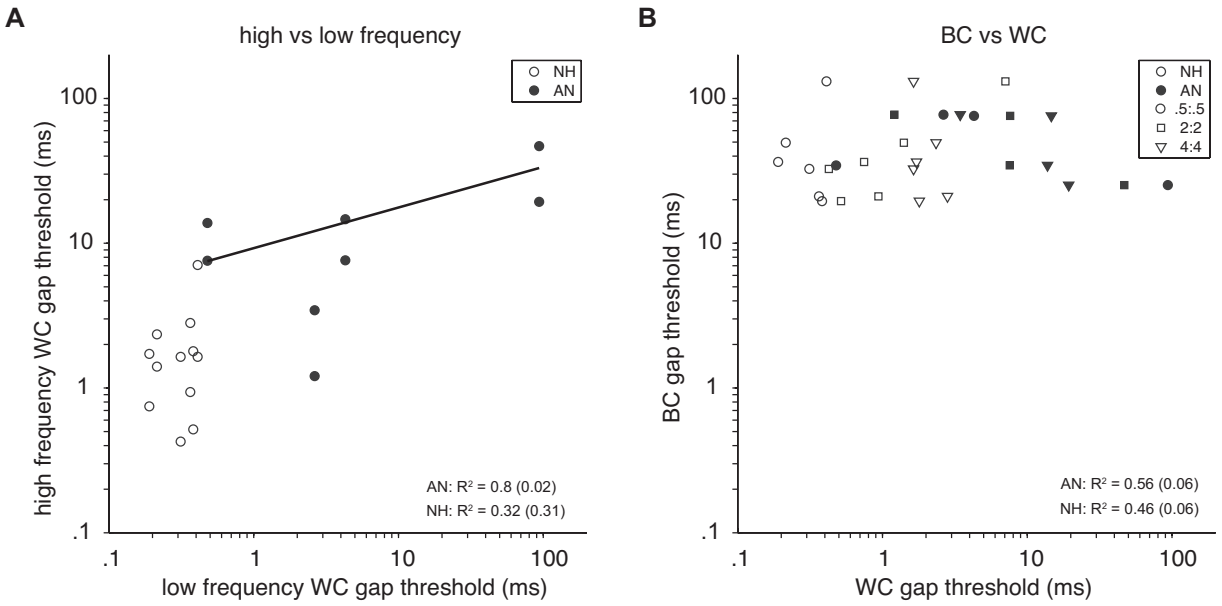

Figure 3.3 Correlation analyses of tonal within- and between-channel gap detection.
Open and filled symbols indicate data from normal hearing (NH) and auditory neuropathy (AN) groups, respectively. Three within-channel (WC) conditions were tested using .5:.5 (circle), 2:2 (square), 4:4 kHz (triangle). One between-channel (BC) condition was tested using 4:2 kHz. WC gap threshold represents the duration of a detected gap bounded by the same frequency whereas BC gap threshold represents the duration of a detected gap bounded by different frequencies. Each WC condition was paired with the BC condition. A Low frequency (.5 kHz) WC gap thresholds were paired with high frequency (2, 4 kHz) WC gap thresholds. High frequency thresholds significantly correlated with low frequency thresholds in the WC condition. B BC gap thresholds (4:2 kHz) were paired with WC gap thresholds (.5:.5, 2:2, 4:4 kHz) yielded no correlation in both groups.

To determine whether degree of hearing loss, stimulus presentation level, and marker frequency confounds within frequency gap detection we performed additional correlation analyses. Fig 3.4A shows WC gap thresholds as a function of pure tone average (dB Hearing Level). The gap thresholds within frequency positively correlated with degree of hearing loss which accounted for 73% of variance in the auditory neuropathy subjects (p = 0.01). Normal hearing data showed no correlation (R^2 = 0.08, p = 0.75). Considering higher degree of hearing loss predicted longer WC gap thresholds, we examined the effect of presentation level. Fig 3.4B shows gap thresholds within frequency as a function of

stimulus level in the auditory neuropathy group. Stimulus level failed to correlate with WC gap thresholds, which argues against presentation level as a confounding factor. Considering that higher frequency usually correlates with more hearing loss, we examined correlations between WC gap thresholds and marker frequency (Fig 3.4C). Gap thresholds within frequency had no correlation with marker frequency in both groups (AN: $R^2 = 0.19$, $p = 0.55$; NH: $R^2 = 0.42$, $p = 0.09$). Thus, we argue against hearing loss associated with higher frequency as confounding factors in WC gap thresholds. Gap thresholds for gaps within frequency tended to be longer with higher frequency markers (Fig 3.4C).

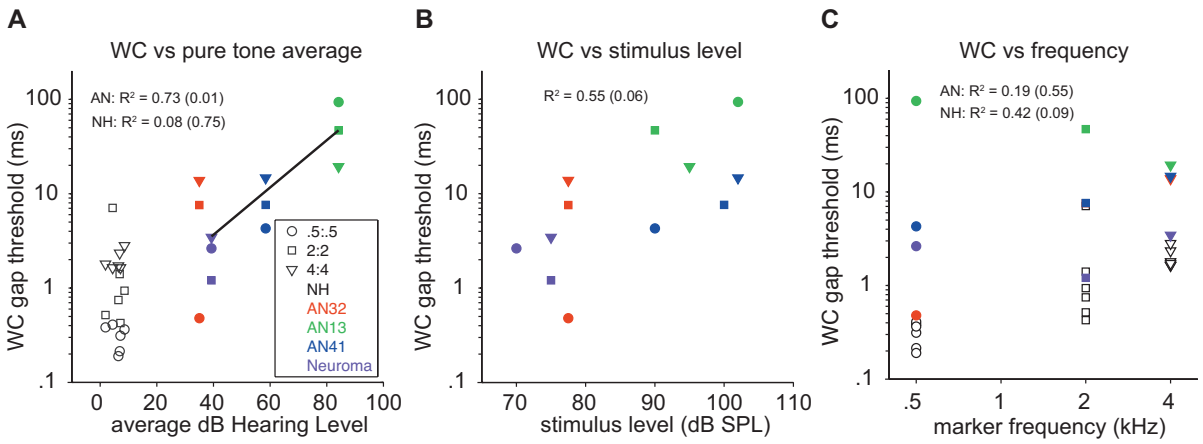

Figure 3.4 Correlation analyses of tonal within-channel gap detection and degree of hearing loss, stimulus level, and frequency. Open and filled symbols represent subjects from normal hearing (NH) and auditory neuropathy (AN) groups, respectively. Different colors denote different subjects in the AN group. Three within-channel (WC) conditions were tested: .5:.5 (circle), 2:2 (square), 4:4 kHz (triangle). WC gap threshold represents the duration of a detected gap bounded by the same frequency. **A** Pure tone average (dB Hearing Level) was calculated across octave frequencies .125-12 kHz and between ears. WC gap thresholds correlated significantly with degree of hearing loss. **B** Gap stimuli were presented at each AN subject's most comfortable loudness level. WC thresholds appear to approach a near significant correlation with increasing stimulus level. **C** WC gap threshold had a non-significant correlation with marker frequency in both groups.

DISCUSSION

Our present findings seem to support the hypothesis that neural dis-synchrony and afferent degeneration manifested by auditory neuropathy limits peripheral temporal acuity within frequency. The unexpectedly finding that auditory neuropathy produces normal central temporal acuity between frequency disproves part of our hypothesis. The degree of hearing loss correlates significantly with gap thresholds within frequency, which supports the theory that WC gap detection involves and is limited by a peripheral mechanism potentially associated with impaired inner hair cell transmitter release and/or nerve impulse generation in the eighth nerve dendrites (Starr et al., 1998). We expected greater hearing loss might necessitate higher stimulus levels at higher frequencies, but lack of correlations with WC gap thresholds argue against these confounding effects. In the auditory neuropathy group, WC gap thresholds correlate but BC performance fail to predict WC gap thresholds. These results suggest separability of the mechanisms mediating gap detection within frequency and between frequencies (Phillips & Smith, 2004).

Our findings suggest impaired WC gap detection in the subjects with auditory neuropathy reflect smearing of the temporal structure of neural spike trains normally synchronized to low frequency stimuli. As a result of disrupted neurotransmitter release at the ribbon synapse or demyelinated nerve fibers, we think auditory nerve fibers cannot phase-lock to 5 kHz and 2 kHz stimuli to process dis-continuity in the ongoing discharge rate evoked by the gap stimulus (Zhang et al., 1990). Instead, imprecise latency of discharge to the onset of the leading and lagging marker may account for impaired 4:4 kHz gap detection (Kiang et al., 1965). In auditory neuropathy, the slower decay of sensation during the gap could prevent the silent interval from being detected (Plomp, 1964).

Elevated WC gap detection in subjects AN32 and AN33 with ribbon synapse disorder support our argument. In the NH auditory system, a larger size neural population represent low than high tonal frequencies. Subject AN32 might have a sufficient number of neural fibers and/or number of synchronous nerve fibers to support WC detection at 5 kHz but insufficient at 2 and 4 kHz (Chambers et al., 2016). Gap processing deficits in Subjects AN13 and AN41 with peripheral neuropathy of unknown etiology but presumably a neural disorder might relate to demyelination of the auditory nerve. At this time we do not know the site of demyelination but speculate that it could be in the "peripheral" or Schwann cell myelinated portion of auditory nerve and/or in its "central" portion where the axons are myelinated by oligodendroglial cells (Starr et al., 1998). We postulate pressure from the tumor in the subject with acoustic neuroma may obstruct blood supply to both nerve terminals and inner hair cells leading to not only complete loudness adaptation (Wynne et al., 2013) but also gap processing deficits in the .5 and 4 kHz but not in the 2 kHz region. Larger variability in WC gap thresholds can reflect dispersed temporal processing in subjects with auditory neuropathy as compared to normal controls.

Normal BC gap detection in the auditory neuropathy group suggests intact central processes for BC gaps that are not limited by disrupted auditory nerve activity. Disorder of the auditory nerve seems to limit WC gap processing on the order of several milliseconds whereas central computations limit BC gap processing on the order of hundreds of milliseconds. It remains unclear why cross-correlation of the activity in two different channels produce poorer temporal acuity than discontinuity detection in any single channel (Phillips et al., 1997). One explanation could involve shifts of attentional processes from the perceptual channel activated by the leading marker and the subsequent timing

consuming process of shifting to those processing the channel representing the trailing marker (Fitzgibbons et al., 1974). We propose that BC gap stimuli stimulate a broader distribution of auditory nerve fibers which do not overlap therefore a subject could detect BC gaps using multiple perceptual channels. In contrast, detecting WC gaps depend on synchronous firing of overlapping auditory nerve fibers limited to one perceptual channel. Synaptic or neural disorders produce a temporal delay on the order of tens of milliseconds, but central mechanisms remain unaffected on the order of hundreds of milliseconds. Forward masking of single auditory nerve fiber responses may relate to the mechanism for WC gap detection. Forward masking describes an effect whereby the magnitude of firing rate evoked by one stimulus reduces the magnitude of discharge rate evoked by a second stimulus that follows (Harris & Dallos, 1979). One can think of gap detection as a process of detecting the burst of spikes to the onset of the sound after the gap. Auditory neuropathy subjects may poorly detect a gap between two identical tones due to a larger than normal effect of forward masking of a single fiber's responses and in turn greater attenuation of the responses evoked by the second tone. Hence, we reason that the forward masking single-fiber mechanism could be disrupted with auditory neuropathy without having much effect on the central process. Different peripheral and central contributions to WC and BC gap detection, respectively, suggest separate neural mechanisms.

The frequency effect we observed in tonal WC gap detection differs from other reports of gap detection in noise centered on low frequencies. Gap detection using narrow-band signals centered on frequencies less than 0.5-1.0 kHz exceed that at higher frequencies (Hall et al., 1996). There are two plausible reasons: 1) low center-frequency noise signals have low-frequency fluctuations in amplitude that can obscure the perceived

gap and 2) relatively narrow cochlear filter for low center frequencies and associated "ringing" at the offset of the leading marker consequently can partially fill the gap (Moore et al., 1993). We propose two explanations for our result: 1) larger cochlear area and corresponding neural population representing low than high tonal frequencies facilitate low frequency gap detection especially at higher sensation levels which excite more nerve fibers and 2) the pink noise masks more low frequency including "ringing" at the leading marker offset to improve gap detection.

Future directions include increasing power of the sample size of auditory neuropathy subjects. Recruiting a subject with cochlear sensorineural hearing loss to serve as a control for hearing loss could help addressing potential confounding effects on gap detection (Zeng et al., 1999; Wynne et al., 2013). Further investigation of between-channel gap detection at different sensation levels (Zeng et al., 1999; Zeng et al., 2005) could determine whether reduced peripheral input affects central temporal acuity.

In summary, differences in WC and BC gap detection may help differentiate peripherally- vs. centrally-based temporal processing disorders.

Author Contributions

Conceived and designed the experiments: CQP and FGZ. Performed the experiments: CQP. Analyzed the data: CQP. Designed the software used for psychophysical testing: CQP.

SUMMARY & CONCLUSIONS

Different sources of hearing impairments arising from loss of sensory inner hair cells, loss of auditory nerve conduction or transmission, or potential loss of nAChRs can similarly contribute to the inability to process cues critical for understanding speech in the presence of competing sounds. The present dissertation includes three studies which investigated the diverse perceptual consequences of peripheral and neural deficits on central auditory processing.

In Chapter 1, our results support the hypothesis that post-lingually deaf cochlear implant (CI) listeners can retain central processing abilities limited largely by poor peripheral encoding. Central processing of temporal cues provided by signal-masker asynchrony remained intact in all CI listeners, however, processing of spectral-variance cues provided by varying intra-cochlear electrodes were preserved in only 2/8 CI listeners. Our acoustic simulation of implant listening support the hypothesis that broad peripheral filters modeling CI-induced current spread which excite non-specific auditory nerve fibers limits utility of spectral-variance cues. In addition to spectral smearing effects, markedly reduced CI dynamic hearing range compared to that in acoustic hearing has negative implications in speech perception. Future experiments may simulate and test reduced dynamic hearing range effects using stimuli with a limited number of discrete loudness steps across the acoustic dynamic range or by adding additional broadband noises to compress the stimuli into a limited acoustic dynamic range. Increasing frequency selectivity (narrower peripheral filters) and dynamic range providing better electro-neural

interface for current generation implants might enable CI listeners better access to spectral-variance cues in speech in a noisy background (Evans et al., 2009; Leake et al., 2011; Middlebrooks & Snyder, 2007).

In Chapter 2, we found oral nicotine (6 mg) has no significant effects on central gain (tone-in-noise detection), temporal acuity (gap detection), or frequency resolution (ripple discrimination) due largely to maximal performance. Variability in gap processing, however, between frequencies at low sound level just reached a significant decrease with nicotine which could reflect temporal summation of cortical responses (Kawai et al., 2007). Reaction times, error rates, and derived attention measures for discriminating two tones using frequency or ear of presentation cues show non-significant drug effects. In general, low task demand and high individual baseline attentional processing seem to stunt nicotine effects. Our correlation analyses propose separability of attention and auditory processes. In particular, no correlation suggests distinct gap detection mechanisms for within- and between-channel cases. Future studies may examine nicotine effects in clinical populations with lower attentional processing (e.g., Alzheimer's, Levin et al., 2006; Sarter et al., 2009; central auditory processing disorders, Musiek et al., 2010) using (titrated) transdermal nicotine (Newhouse et al., 2012) or oral nicotine to study effects on the central auditory processing.

Lastly, in Chapter 3, our data support the hypothesis that neural dis-synchrony due to auditory neuropathy limits peripheral temporal acuity on the order of several milliseconds but, surprisingly, not central temporal acuity on the order of hundreds of milliseconds. The degree of hearing loss correlates significantly with gap thresholds within frequency supporting a potential peripheral mechanism associated with ribbon synapse

transmitter release and/or nerve impulse generation (Starr et al., 1998) mediates within-channel gap detection. Positive correlation analyses suggest separability of the mechanisms mediating gap detection within frequency and between frequencies (Phillips & Smith, 2004). Immediate future directions included increasing power of the auditory neuropathy sample size, and recruiting a control subject with cochlear sensorineural hearing (Zeng et al., 1999; Wynne et al., 2013). Future experiments testing different within- and between-channel gap detection at different sensation levels may reveal temporal deficits due to reduced auditory ganglion cell input to the brain. Differences in within- and between-channel gap detection may be used clinically to differentiate temporal processing disorders with peripheral vs. central origin.

Studying perceptual effects of peripheral and neural deficits in central auditory processing provide unique insights into the marvelous complexity in processing between the ears and brain.

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APPENDIX A: Effects of nicotine, nicotine agonists, and antagonists on attention mediated sensory processing

Reference	Subject	Treatment	Task	Effect
Rezvani & Levin 2003	Rats	Nicotine	Operant visual signal detection	+ Increase percent correct rejection and increase choice accuracy suggesting improvement in attention
Stolerman et al., 2000	Rats	Nicotine	5-choice serial reaction task[a]	+ Dose-related increase in accuracy of completed trials (correct responses) and decrease in omission errors and reaction times
Terry et al., 2002	Rats	SIB-1553A[b] (nicotinic agonist)	5-choice serial reaction task	-/+ SIB-1553A did not improve performance in normal rats, but did reverse attention deficits induced by addition of distracting stimuli and by the NMDA-sensitive glutamate receptor antagonist, dizocilpine
Levin et al., 2014	Rats	DHβE[c] & MLA[d] (nicotinic antagonists)	Operant visual signal detection	+ DHβE improves attention accuracy either alone or in reversing dizocilpine-induced attention impairment; MLA significantly attenuates the dizocilpine-induced attentional deficits
Le Houezec et al., 1994	Humans (non-smokers)	Subcutaneous nicotine injection (0.8 mg)	Choice reaction time task[e]	+ Low dose of nicotine directly affects attention or stimulus processing components of information processing
Lawrence et al., 2002	Humans (smokers)	Transdermal nicotine patch (21 mg)	Rapid visual information-processing task	+ Increase task-induced activation in parietal cortex, thalamus, and caudate with nicotine treatment suggest nicotine-induced improvement in attention
Harkrider & Hedrick, 2005	Humans (smokers & non-smokers)	Transdermal nicotine patch: 21 mg (smokers) 7 mg (non- smokers)	Consonant vowel (CV) discrimination in quiet and in broadband noise	+ All subjects demonstrate nicotine-enhanced behavioral and electrophysiological CV discrimination in noise

[a]Rodent model of attention that requires detection of a signal light presented randomly in one of five locations during 30-min sessions (Robbins, 2002); [b]SIB-1553A, (±)-4-{[2-(1-methyl-2-pyrrolidinyl)ethyl]thio}-phenol hydrochloride (hippocampal and prefrontal cortical β4 nAChR agonist); [c]DHβE, dihydro-β erythroidine (hippocampal α4β2 nAChR antagonist); [d]MLA, methyllycaconitine (hippocampal α7 nAChR antagonist); [e]Stimulus Evaluation- Response Selection task used to discriminate between two processing stages— stimulus evaluation and response selection (Callaway et al. 1985; Naylor et al. 1985). Net effect on performance: + indicates improvement, - indicates impairment

APPENDIX B: Heath and Tobacco Use Screening Test

Reference: modified from Table 4 in Bramer SL, Kallungal BA (2003) Clinical considerations in study designs that use cotinine as a biomarker. Biomarkers. 8:187-203.

Do you have any history of hearing loss or dysfunction? (i.e. ringing in the ears, use of hearing aids, sensitivity to sounds, single-sided deafness, etc)

Do you have a personal health history of any of the following?

□ Diabetes mellitus
□ Renal failure
□ Cardiovascular disease
□ Neurological disease
□ Drug/ Alcohol dependency
□ Mental illness
□ Central Nervous System disorder
□ Regular use of prescription medications (excluding oral contraceptive)
□ None of the above

Do you smoke? If so, in what form? (e.g. cigarettes, e-cigarettes, hookah, pipes, cigars, etc.) Check all that apply.

□ Cigarettes
□ E-cigarettes
□ Hookah
□ Pipes
□ Cigars
□ I do not smoke
□ Something not listed above (indicate below)

Other (please specify)

Do you smoke <4 cigarettes in a month (or <1 cigarette per week)?

□ Yes
□ No
□ I do not smoke

Have you smoked more than 100 cigarettes in your lifetime and any in the past year?

□ Yes
□ No

Are you taking blood pressure medication or other prescribed drugs?

□ Yes
□ No

Is English your first language?

□ Yes
□ No

□ Learned English and another language simultaneously FROM BIRTH

Are you between the ages of 18-60?
□ Yes
□ No

Are you exposed to tobacco products by handling them (e.g. farming tobacco, manufacturing cigars, etc.)?
□ Yes
□ No

Do you use tobacco products of any form (e.g. cigarettes, pipes, cigars, chewing tobacco, etc.)? Check all that apply.
□ Cigarettes
□ Pipes
□ Cigars
□ Chewing tobacco
□ I do not use tobacco products
□ Something other than listed above (indicate in box below)
□ None of the above
Other (please specify)

Approximately how many cigarettes, cigars, or pipes do you smoke per day? (e.g. >1 cigarette per week or 4 per month?)

Are you an ex-smoker? If so, about when [month/year] did you quit? Have you used tobacco products within the last 6 months?

Do you use any products to help quit or prevent smoking (e.g. gum, patch, etc.)? Do you live with a smoker?

Have you been around any second-hand smoking in the past 3 days prior to testing? (e.g. bar, restaurants, etc.) If so, for how long?

Are there any circumstances where you are routinely (at least once a day) exposed to 'second hand' smoke?

Have used any other recreational drugs (i.e. THC) in the last 3 days prior to the experiment?
□ Yes
□ No

Are you able to refrain from smoking or consuming tobacco products within 3 days before testing?
□ Yes
□ No

APPENDIX C: Fagerström Test for Nicotine Dependence

Reference: Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO (1991) The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. Br J Addict 86:1119–1127.

How soon after you wake up do you smoke your first cigarette?
□ After 60 minutes (0)
□ 31-60 minutes (1)
□ 6-30 minutes (2)
□ Within 5 mintutes (3)

Do you find it difficult to refrain from smoking in places where it is forbidden?
□ No (0)
□ Yes (1)

Which cigarette would you hate most to give up?
□ The first in the morning (1)
□ Any other (0)

How many cigarettes per day do you smoke?
□ 10 or less (0)
□ 11-20 (1)
□ 21-30 (2)
□ 31 or more (3)

Do you smoke more frequently during the first hours after awakening than during therest of the day?
□ No (0)
□ Yes (1)

Do you smoke even if you are so ill that you are in bed most of the day?
□ No (0)
□ Yes (1)

Your level of dependence on nicotine is (add up the numbers in the parentheses):
□ 0-2 Very low dependence
□ 3-4 Low dependence
□ 5 Medium dependence
□ 6-7 High dependence
□ 8-10 Very high dependence

APPENDIX D: Mood Scale & Symptoms Rating

Mood ratings

Reference: Harkrider AW, Hedrick MS (2005) Acute effect of nicotine on auditory gating in smokers and non-smokers. Hear Res 202:114-128.

Status	Mood	strongly 0	slightly 1	neither 2	slightly 3	strongly 4
pre trt	tense/relaxed					
pre trt	nervous/calm					
pre trt	energetic/tired					
pre trt	alert/drowsy					
pre trt	content/irritated					
pre trt	satisfied/dissatisfied					
pre trt	focused/distracted					
pre trt	happy/depressed					
pre trt	hungry/satiated					
post trt	tense/relaxed					
post trt	nervous/calm					
post trt	energetic/tired					
post trt	alert/drowsy					
post trt	content/irritated					
post trt	satisfied/dissatisfied					
post trt	focused/distracted					
post trt	happy/depressed					
post trt	hungry/satiated					

Side Effects

Reference: Parrott AC, Garnham NJ, Wesnes K, Pincock C (1996) Cigarette Smoking and Abstinence: Comparative Effects Upon Cognitive Task Performance and Mood State over 24 Hours. Hum Psychopharmacol 11:391–400.

None	Mild	Moderate	Mod-severe	Severe
1	2	3	4	5

Mild: slightly jittery
Moderate: jitter, dull headache
Moderately severe: jittery, headache, nausea
Severe: jittery, pounding headache, nausea, vomiting

"In summary, there are no small problems. Problems that appear small are large problems that are not understood… Nature is a harmonious mechanism where all parts, including those appearing to play a secondary role, cooperate in the functional whole."
- Santiago Ramón y Cajal, *Advice for a Young Investigator*