INFERENCE OF PHYSIOLOGIC PROCESSES USING OTOACOUSTIC EMISSIONS

By

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Presented to the Department of Biomedical Engineering
and the Oregon Health & Science University
School of Medicine
in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy
in
Electrical Engineering

December 2010

Oregon Health & Science University

School of Medicine

Department of Biomedical Engineering

CERTIFICATE OF APPROVAL

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Acknowledgments

This dissertation research represents the hard work and dedication of numerous people all of whom have helped in one way or another. Without the help of my friends, family and colleagues, I could not have completed this body of work.

First and foremost, I would like to thank my wife, Elizabeth O'Neill, for her strong support, unfailing confidence in me, and valuable comments, critiques and feedback throughout the entire process of this dissertation.

I would like to thank my advisor and mentor, Dr. Eric A. Wan, for encouraging the pursuit of funding of this project in the earliest stages before pilot data had been taken and for his ongoing support of the research itself through his suggestions, critiques, and superb technical insights that made this research possible.

I am grateful for the help of Dr. Dawn Konrad-Martin for being an outstanding hearing science mentor for me within this work, for providing the data set that was used in chapter 4, and for being a member of my dissertation committee. I would like to extend thanks to Dr. Misha Pavel and Dr. John-Paul Hosom for serving on my dissertation committee and taking the time to provide valuable feedback and criticisms that have ultimately made me a better researcher. I am also very thankful for the support of Dr. Jan van Santen for giving me the freedom and support to pursue my own research interests and for providing ongoing tuition reimbursement through the OHSU BME/CSLU department. I am thankful to Dr. Deniz Erdogmus from Northeastern University who provided early feedback on the project and served on my RPE committee. And I greatly appreciate the help of Pat Dickerson in the administrative office of CSLU.

All of my experiments for this research were conducted at the National Center for Rehabilitative Auditory Research (NCRAR) at the Portland VA Medical Center. I am very thankful for the support of my exceptional colleagues at NCRAR and for their assistance along the way. In particular, I would like to thank Dr. Erick Gallun from NCRAR who helped in providing feedback in the design of the experiment described in chapter 3. I would like to offer a very special thanks to Dr. Stephen Fausti, the director of the NCRAR. Without the support of Dr. Fausti and the NCRAR, we would not have had the facilities, personnel, or deep knowledge base necessary to carry out the research projects described in this dissertation. I would like to thank Garnett McMillan for his suggestions on statistical modeling approaches. I appreciate the support from Dr. David Kagen for serving as the clinical PI for the project at the Portland VA Medical center. Thanks to Dan McDermott and Kara Schvartz for their assistance with data acquisition and patient testing. Many thanks to Patrick and Wendy Helt for the administrative support at the Portland VA Medical center, and for help with grant submissions.

I would also like to thank my many colleagues who provided feedback on my work through discussions at conferences. Comments and criticisms of my work made all the difference in helping me fully develop my ideas. I'd specifically like to thank Dr. Sumit Dhar and Dr. Doug Keefe for their expertise on OAEs and their advice and encouragement during my training. And I'd like to thank Dr. John Guinan Jr. and his research group at Harvard Medical School for taking the time to review my results and provide valuable feedback on my data.

I would also like to thank Dr. Erik Cheever and Dr. Kathleen Siwicki from Swarthmore College who were early mentors for me and who have always encouraged me to be a better engineer and scientist.

I am very appreciative of the generous funding support we received for this work, without which none of this research could have been done. Specifically, we received an R21 grant from NIH / NIDDK (1R21 DK079283-01A1) and support from the NCRAR center grant (VA RR&D C2659C).

And finally, I would like to thank my mother, Janis Daly, my father Gordon Jacobs, my sisters Tonya Jacobs, Kori Irwin, Aimee Jacobs, my stepfather Dennis Daly and stepmother Rie Jacobs and my two children Dylan and Ingrid for their steady love and support throughout my life.

Abstract

Inference of physiologic processes using otoacoustic emissions

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This dissertation research is focused on developing new noninvasive methods of inferring physiologic processes using features of otoacoustic emissions (OAEs). The physiologic processes that were studied include blood glucose levels, focused auditory attention, and age-related hearing changes. Current state-of-the art methods for inferring such physiologic processes are invasive and oftentimes painful, time-consuming and labor-intensive, or non-existent. OAEs are low intensity sounds generated by the cochlea in response to acoustic stimuli. Evoking and measuring an OAE is done using a tiny speaker and microphone that fit snugly inside the ear canal. The OAE response can be partially inhibited or reduced in amplitude by presenting competing acoustic stimuli contralaterally (opposite ear), ipsilaterally (same ear), or both. This inhibition effect is caused by activation of the medial olivocochlear (MOC) efferent nerve pathways from the brain. In this dissertation, results are presented that show how stimulus frequency (SF) OAE amplitudes and phase as well as MOC-related changes in SF OAEs correlate with blood sugar levels. A mechanism is proposed whereby blood glucose acts (1) as a metabolic mediator of the endocochlear potential and (2) as a neurotransmitter by being transformed to ATP and acting on ATP-sensitive ion channels in the cochlear outer hair cells; a process which is mediated by calcium release by activation of the MOC. A new prediction methodology is described that is called Hyperglycemic Risk Analysis (HRA) in which elevated blood sugar levels are predicted using metrics from SFOAEs recorded with and without activation of the MOC. Leave-one-out cross-validated receiver operating characteristic (ROC) analysis showed HRA

achieves good accuracy with an area-under-the-curve (AUC) of 0.85. Clinical applications for HRA could ultimately lead to a method for noninvasively monitoring elevated glucose levels in diabetes patients. Also included within this dissertation are results from an experiment in which we investigated how focused attention influences MOC inhibition. Results are presented from an experiment in which test subjects simultaneously had their MOC activated while they performed psychoacoustic listening tasks that required varied levels of focused attention. Features within the MOC inhibition were analyzed relative to the psychometric functions to infer effect of focused attention on the MOC. A statistically significant increase in the MOC inhibition amplitude was observed when the subjects were actively participating in the listening task compared with when the subjects were passively listening. Finally, this dissertation presents results demonstrating how aging influences the auditory system and specifically SF OAE amplitude and latency. Results from a 53-subject study are presented that show how SF OAE amplitudes can be used to predict age-related hearing changes to the auditory system. A new relationship between SF OAE probe frequency and suppressor level are also presented and a corresponding model is described. Results are presented as they relate to proposed extensions to a current auditory model.

Chapter 1

1. Introduction

The overall objective of this dissertation research was to investigate the relationship between otoacoustic emissions (OAEs) and several physiologic processes in humans including blood sugar levels, focused auditory attention, and age-related hearing changes. Patients suffering from diabetes are unable to maintain a constant blood sugar level. Type 1 diabetic patients can suffer from hypoglycemia whereby their blood sugar is too low. Hypoglycemia, if left untreated can lead to feelings of discomfort, seizures, and in rare cases brain damage or death. Type 1 and type 2 diabetes patients can also suffer from hyperglycemia whereby blood sugar goes too high. Long term exposure to hyperglycemia can cause neuropathy, retinopathy, and damage to tissue and organs. To avoid conditions of hypoglycemia and hyperglycemia, diabetic patients are instructed to monitor their blood sugar levels several times each day (American Diabetes Association, 2001). Current invasive methods of monitoring blood glucose levels in diabetic patients involve painful finger sticks. Many diabetic patients fail to actively manage their glucose for the primary reasons of finger soreness, pain, inconvenience, and fear of needles (Burge M. R., 2001). Newer state-of-the art glucose monitoring methods have been developed that enable continuous monitoring of glucose whereby a needle is inserted into the body and blood sugar is available to a patient in real time over the period of 3-7 days (McGarraugh, 2009; Mazze, Strock, Borgman, Wesley, Stout, & Racchini, 2009; Battelino & Bolinder, 2008; Gilligan, et al., 2004). However such continuous monitoring methods are still invasive, they can limit activity of the patients, and their reliability and accuracy are not as robust as finger-stick methods of glucose testing (Mazze, Strock, Borgman, Wesley, Stout, & Racchini, 2009). The primary objective of this dissertation research was to investigate the relationship between OAEs and blood sugar levels such that a new noninvasive method for estimating blood sugar could ultimately be developed. In chapter 2 results are presented from experiments demonstrating how OAEs

modulated by efferent nerve fibers descending through the medial olivocochlear (MOC) bundle change during hyperglycemia. Also in chapter 2, a new method called hyperglycemic risk analysis (HRA) is presented in which we show how hyperglycemia can be estimated using metrics from MOC modulated OAEs. A secondary objective of this dissertation research was to investigate how the MOC activation changes with focused auditory attention. Patients' attention to auditory stimuli during OAE experiments can potentially be a confounding factor, particularly when OAEs are evoked in such a way that MOC efferent nerve pathways descending from the auditory cortex are simultaneously being activated by the auditory stimuli. The MOC is activated by higher level noise stimuli, and it is believed that the MOC is used to improve speech understanding in noisy environments (Kumar & Vanaja, 2004; de Boer & Thornton, 2008). In addition to basic science reasons for investigating cortical control of the MOC, there are practical reasons as well for investigating this topic. Specifically, if cortical control of the MOC can be demonstrated and characterized, new training rehabilitation strategies may ultimately be possible that could be used to improve communication ability in people with hearing impairment. In chapter 3, we show how activation of these MOC efferent nerve fibers changes during active listening tasks compared with passive listening, thereby providing further evidence that the MOC can be cortically controlled. A final objective of this research was to investigate how OAE amplitudes and latencies change relative to age. It is still not known how aging affects the auditory system, as many groups who have studied age do not adequately control for hearing impairment. Current auditory diagnosis and rehabilitation strategies are geared specifically towards people with hearing impairment. However, it may be that people with age-related hearing changes require different diagnosis and rehabilitation strategies than people with acute trauma-related hearing impairment. In chapter 4 results are presented from an experiment that demonstrates how OAE amplitudes decrease with age while OAE latencies remain unchanged with age, independent of hearing impairment. We also present results in chapter 4 that show how

probe and suppressor stimulus levels influence OAE amplitude and latency measures, thereby providing new information about the generation mechanism of OAEs.

OAEs, first reported in humans by Kemp (1978) are low-level sounds emitted from the ear caused by motion of hair cells within the cochlea. OAEs can occur spontaneously (SOAEs) or in response to an audio stimulus (evoked or eOAEs). They can provide a noninvasive test of the cochlear mechanical response to acoustic stimuli and they are already widely used in humans and animals to study cochlear function and hair cell micromechanics in both normal and damaged ears (Berlin, 2002; Kim, 1980; Kim, Molnar, & Matthews, 1980; Zurek, Clark, & Kim, 1982; Kemp, 1986; Shera, Guinan, & Oxenham, 2002). OAEs are currently used clinically to screen for hearing impairment in newborn infants (Meier, Narabyashi, Probst, & Schmuziger, 2004; Robinette, Cevette, & Webb, 2002). OAEs are modulated by efferent nerve pathways that descend from the brainstem, through the medial olivocochlear (MOC) bundle, and onto the cochlear outer hair cells (Guinan Jr. J. , 2006). Activation of these efferent nerve pathways has been shown to reduce the amplitude of OAEs through inhibition of the cochlear amplifier gain (Mountain, 1980; Siegel & Kim, 1982; Collet, Kemp, Veuillet, Duclaux, Moulin, & Morgon, 1990; Guinan Jr. J. , 2006). A primary contribution of this work is to show that the efferent MOC inhibition of the OAE changes during hyperglycemia, and that the OAE amplitude also changes during hyperglycemia. A secondary contribution of this research is that we show that the MOC can be actively controlled by the auditory cortex. And another secondary contribution of this research is a result showing that age impacts the auditory system independent of hearing impairment. Each of these three topics is discussed independently and then a detailed summary of contributions of this work is given.

1.1. Influence of glucose on auditory system

There are two potential mechanisms by which glucose could be affecting OAEs and efferent pathways modulating OAEs. The first potential mechanism is based on the fact that

glucose is the energy source for the cochlea, and if this energy source is disrupted, it affects the function of the cochlea (Mendelsohn & Roderique, 1972; Wing, 1959; Koide, 1958). Wing (1959) and Koide (1958) showed that hypoglycemia caused a reduction in the endocochlear potential (EP). Cochlear microphonics are extracellular receptor potentials, dominated by outer hair cell receptor currents (Davis, Deatherage, Rosenblut, Fernandez, Kimura, & Smith, 1958) and a reduction in cochlear microphonics amplitude presumably is a result of EP drop. A reduced cochlear microphonic amplitude during hypoglycemia may be caused by the lack of available energy required for maintaining the active transport of sodium and potassium ions into and out of the endolymph in the cochlea. Within the endolymph, there is a high concentration of potassium and a low concentration of sodium, as first shown by Smith et al. (1954). This concentration gradient is what is responsible for maintaining the large EP between outer hair cells and the endolymphatic fluid, and when the cochlea is deprived of glucose, the EP drops (Mendelsohn & Roderique, 1972). A reduction in EP has also been shown to cause a reduction in OAE amplitudes (Mills, Norton, & Rubel, 1993). For this reason, we hypothesize that OAE amplitudes will have a positive correlation with blood glucose. While the first mechanism is expected to show a positive correlation of OAE amplitude with blood glucose, the second mechanism is expected to influence the MOC, which has an inhibitory influence on OAE amplitudes. The second potential mechanism is based on the research first presented by Bobbin et al. (1978) that adenosine tri-phosphate (ATP) is not only used for energy in the cochlea, but that it is an important neurotransmitter in hair cells and other supporting cells within the cochlea. ATP is generated when cells break down glucose for energy storage and it has been shown that outer hair cells have ion channel receptors that are sensitive to ATP as a neurotransmitter (Yu & Hong-Bo, 2008; Kujawa, Fallon, & Bobbin, 1994b; Munoz & Thorne, 1995; Munoz, Thorne, & Housley, 1999; Sueta, Paki, Everett, & Robertson, 2003; Bobbin & Thompson, 1978). Kujawa et al. (1994a) demonstrated that when ATP and ATP analogues were applied to the cochlear perilymph, reductions were observed in OAE amplitudes and auditory nerve compound action potentials.

Some of these OAE amplitude reduction effects could be reversed when perfusing the perilymph with ATP antagonists, further supporting the theory that ATP-sensitive ion channels exist in the outer hair cells (Kujawa, Fallon, & Bobbin, 1994b). Importantly, it has been shown that calcium ions (CA^{++}) are necessary for ATP-induced effects to occur (Yu & Hong-Bo, 2008). The dependence of the ATP-induced effects on calcium is relevant to the MOC because the efferent neurotransmitter acetylcholine (ACh) is released by the MOC terminals and acts on $\alpha 9\alpha 10$AChRs subunit terminals that allow entry of Ca^{++} ions into the outer hair cell (Elgoyhen, Vetter, Katz, Rothlin, Heinemann, & Boulter, 2001; Guinan Jr. J. J., 2010). The MOC is known to exhibit an inhibitive effect on OAE amplitudes and this same inhibitive effect has been demonstrated by perfusion of the cochlea with ATP and ATP agonists. Since ATP impacts on OAEs is reliant on Ca^{++}, and since CA^{++} is released by MOC activation, this is a possible mechanism for how ATP-related inhibition of OAEs could be modulated by MOC activation. Results from this research lead us to hypothesize that (1) while OAEs might be larger in amplitude in hyperglycemic conditions due to an acute increase in the EP, (2) the MOC inhibition of OAEs will also increase during hyperglycemic conditions. The primary contribution of this work is based on the discovery that both OAE amplitudes and MOC inhibition amplitudes increase with hyperglycemia consistently across subjects. These results are presented in chapter 2. A new method for predicting hyperglycemia using metrics from OAEs suppressed by MOC activation called hyperglycemic risk analysis (HRA) is also discussed in chapter 2.

1.2. Influence of auditory attention on MOC

A secondary objective of this dissertation research is to investigate whether the auditory cortex controls the MOC to determine what effect this may have on measurements taken during the glucose / MOC correlation experiments. While the MOC has been actively studied by a number of research groups, it is still not known whether the MOC activation is a lower-brainstem reflex or alternatively controlled by the auditory and/or visual cortex. There is significant

evidence from anatomical studies and animal experiments leading to the hypothesis that the MOC is controlled by the cortex (Mulders & Robertson, 2002; Hernandez-Peon, 1966; Hernandez-Peon, Scherrer, & Jouvet, 1956; Perrot, et al., 2006). And certain human studies in which epileptic subjects' auditory cortex was electrically stimulated during surgery demonstrated a reduction in OAE amplitude similar to what is observed during MOC activation (Perrot, et al., 2006). There has also been work examining the effect of active vs. passive listening on MOC inhibition amplitudes. Maison et al. (2001) found that when attention was given to a certain frequency in the contralateral ear, MOC inhibition of OAEs evoked at that same frequency were larger in amplitude compared with when the frequencies in the ipsilateral and contralateral ear were different. Harkrider and Bowers (2009) did an experiment in which they investigated passive vs. active listening tasks and found that MOC inhibition decreased during active listening, seemingly contradicting Maison's work. De Boer and Thornton (2007) also found that active attention leads to a smaller MOC inhibition effect. This discrepancy in the literature led us to investigate the topic ourselves. We were particularly interested because cortical control over the MOC could potentially impact results of glucose / OAE correlation testing if the test subject is actively controlling the MOC response through their attention to auditory stimuli. A secondary contribution of this dissertation research given in chapter 3 demonstrates that there is a statistically significant increase in MOC inhibition amplitude during an active listening task in which the subject is instructed to identify a target probe tone in noise as compared with when the subject is instructed to be a passive listener and do nothing during the MOC recordings. We also show in chapter 3 how these results do not contradict the earlier work and how the different papers written on this topic complement each other.

1.3. Influence of aging on auditory system

A final objective of this dissertation research is to investigate how aging could be influencing the auditory system and specifically the generation mechanism of stimulus frequency

OAEs. The research question to be answered is specifically as follows: how do OAE amplitudes and latencies change with aging, and are the changes independent of hearing impairment? While it is commonly known that hearing performance deteriorates with aging (Davis, Ostri, & Parving, 1990; Gates, Cooper, Kannel, & Miller, 1990; Ostri & Parving, 1991; Divenyi, Stark, & Haupt, 2005), it is more difficult to assess whether the decline in hearing performance is due to acute damage from noise exposure or due to natural changes in the auditory system due to aging. OAE amplitudes have been shown by many groups to decrease in amplitude with aging, but results have been contradictory with some arguing that hearing impairment has not been properly controlled for in these experiments (Strouse, Ochs, & Hall, 1996; Dorn, Piskorski, Keefe, & Neely, 1998; Chida, 1998; Stover & Norton, 1993; Bonfils, Bertrand, & Uziel, 1988; Collet, Moulin, & Morgon, 1990; Hoth, Gudmundsdottir, & Plinkert, 2010). Few have reported on OAE latency changes with aging (Ramotowski & Kimberley, 1998). OAE latency has been proposed to be an indicator of cochlear tuning based on the assumption that the OAE generator mechanism near the tonotopic place related to the stimulus acts like a minimum phase filter (Shera, Guinan, & Oxenham, 2002). The delays imposed by such filters are approximately proportional to the inverse of the filter bandwidth. It is not known whether aging effects alone impact tuning independent of hearing threshold. We present results in chapter 4 that show that when hearing impairment is controlled for, there is still a consistent and statistically significant drop in OAE amplitude for older subjects. There is no significant change in OAE latency with aging, suggesting that tuning of the auditory system is not something that changes with aging when hearing impairment is controlled. Also in chapter 4, results are presented from a statistical model that quantifies how stimulus levels (probe and suppressor level) affect OAE amplitude and latency.

1.4. Summary of research objectives and contributions

Three overall research objectives are discussed in this dissertation. (1) The primary objective of this research is to demonstrate the correlation between OAEs and the MOC efferent pathway with blood sugar in both diabetic and non-diabetic patients and to use OAEs and MOC metrics to predict blood sugar noninvasively. (2) A secondary objective of this research is to investigate whether the MOC can be controlled by the auditory cortex. (3) And a final objective of this research is to investigate how aging impacts the auditory system independent of hearing impairment.

The specific contributions of this body of research are summarized as follows. Contributions are categorized according to the specific research objectives.

Primary Objective: Correlation of OAEs and MOC inhibition of OAEs with blood glucose

- We present results that show that the amount of MOC inhibition of OAEs in diabetes subjects increases in a consistent, systematic, and repeatable way during hyperglycemia. OAE amplitudes, in the absence of MOC activation also increase during hyperglycemia in most diabetes subjects tested.
- We propose a biological mechanism explaining why increases in OAE and MOC inhibition of OAE amplitude were observed during hyperglycemia. The mechanism explaining the positive correlation of OAE amplitude with blood glucose is based on metabolic influences of blood sugar on the endocochlear potential and cochlear microphonics. The mechanism explaining the positive correlation of MOC inhibition of OAEs with blood glucose is based on the fact that (1) blood sugar is converted to ATP which acts as a neurotransmitter on ATP-sensitive ion channels localized to the base of the outer hair cells and (2), this influence is calcium dependent and finally (3) the MOC

activation results in the release of an efferent neurotransmitter that causes increased extracellular calcium release.

- A new method is presented for predicting hyperglycemia using metrics from MOC inhibition of OAEs and phase waveforms that is called Hyperglycemic Risk Analysis (HRA). HRA demonstrates that prediction elevated blood glucose may eventually be possible in a real-world setting.
- HRA can be extended to include mixed effects accounting for subject variability; prediction accuracy using leave-one-out cross-validated receiver operating characteristic (ROC) analysis yields an area under the ROC curve of 0.85.
- We show that prediction of continuous blood glucose is possible, although accuracy is generally less accurate than current off-the-shelf enzyme-based sensors. Clarke error grid analysis on our methods yields a clinical accuracy rate of 90.2% of data within the A+B region and an r^2 of 0.31 ($p<0.01$). However, only 32% of data fell within the A region and the relative error was high; MARD of 37.2%

Secondary Objective: Auditory cortex impact on MOC inhibition

- Based on results from the attention correlation study in chapter 3, we present further support for the theory that the auditory cortex mediates the activation of the MOC. MOC inhibition is shown to increase during active attention to an auditory detection task relative to the passive listening condition.

Secondary Objective: Influence of aging on auditory system

- We present results that demonstrate an aging effect on OAE amplitudes that is independent of hearing functionality. OAE amplitudes in older adults are smaller than in younger adults. No change in OAE latency was observed, however, indicating that

tuning (a measure hypothesized to be inversely proportional to OAE latency), is not something affected by aging.

- We present a new model that demonstrates in normal hearing subjects how SF OAE amplitude and latency change relative to probe and suppressor stimulus levels.

Included in the remainder of this introduction is an overview of OAEs and the different types of OAEs that are measured. A discussion is presented regarding why a certain type of OAE was selected for these experiments stimulus frequency (SF) OAEs and an overview for the generation mechanism of SF OAEs is discussed. An overview of auditory models is presented and a discussion of how results from this dissertation could potentially fit into current auditory models is also discussed within the introduction.

In Chapter 2 results from the glucose correlation experiments are presented and the hyperglycemic risk analysis (HRA) predictive model is described. In chapter 3, results from the focused auditory attention experiment are presented. And in chapter 4, results are presented that show aging impacts OAE amplitude and latency. Finally, in chapter 5 we discuss how the various factors; namely blood glucose, attention, and aging, influence each other and how results from these experiments can be used in combination with each other to draw conclusions about how the auditory system and OAEs specifically are altered due to physiologic changes.

1.5. Background on OAEs and generation mechanisms

OAEs are generated by the movement of outer hair cells located along the basilar membrane within the cochlea (Kemp, 1978). They are generated either in a quiet environment in which they occur spontaneously (SOAEs), or alternatively, they can be evoked by presenting the ear canal with an acoustic stimulus (eOAEs). OAEs can be evoked using many different types of audio stimuli including tones, clicks, tone bursts, chirps, and combinations of two or more tones. OAEs are evoked by presenting sounds to a subject's ear through a miniature speaker that fits

snugly within the ear canal. The total pressure within the ear is recorded using a miniature microphone that also fits snugly inside of the ear canal. Sound enters the ear canal and pushes against the tympanic membrane, which moves the malleus, incus and stapes bones as shown in Figure 1.

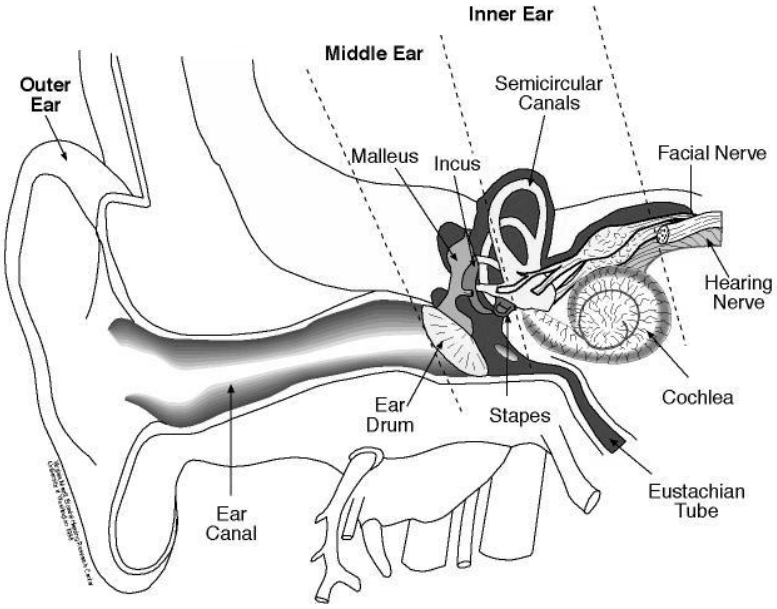

Figure 1: Diagram of the ear (Picture courtesy Kenneth M. Steele, Ph.D.)

The stapes pushes against the oval window membrane of the cochlea. The cochlea is filled with fluid that is non-compressible and since a second membrane, the round window membrane serves as a pressure release port, pressure against the round window pushes fluid, which causes motion of the basilar membrane, which extends along the coiled length of the cochlea as shown in Figure 2.

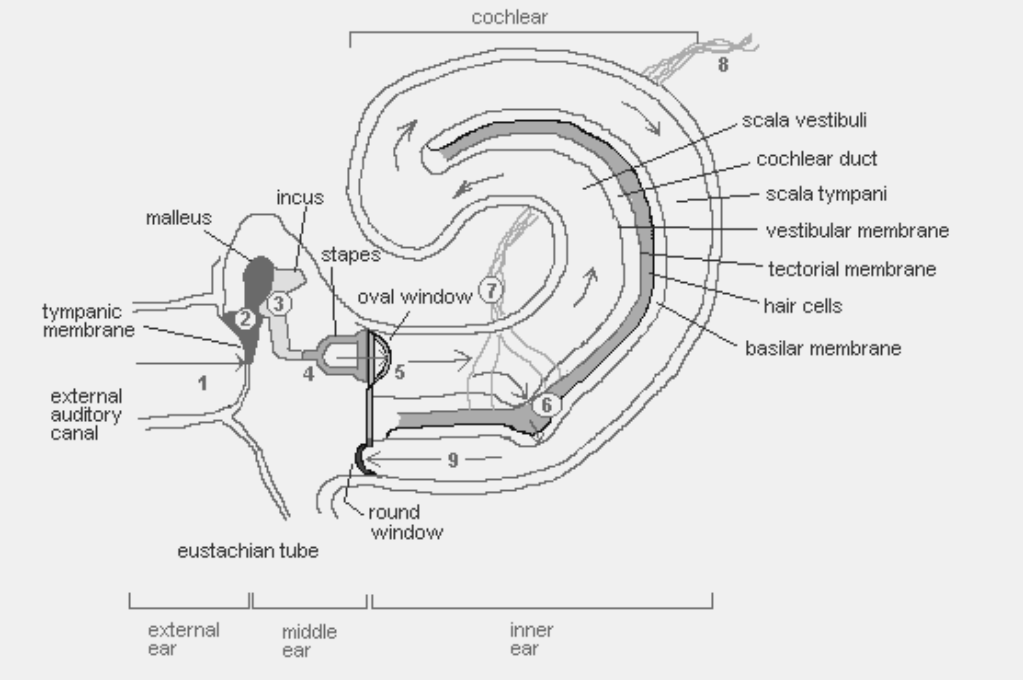

Figure 2: Diagram of ear canal showing details of inner ear. Photo courtesy of Dr. Kenneth M. Steele, Ph.D.

When sound enters the inner-ear, a forward traveling wave is generated on the basilar membrane that begins moving apically. The peak of the traveling wave is thought to be the tonotopic region of SF OAE generation, as it is where a reflected (reverse traveling) wave is generated that is the correct phase to add coherently with the forward traveling wave. Located along the length of the basilar membrane are both inner and outer hair cells. An example of a hair cell is shown in Figure 3.

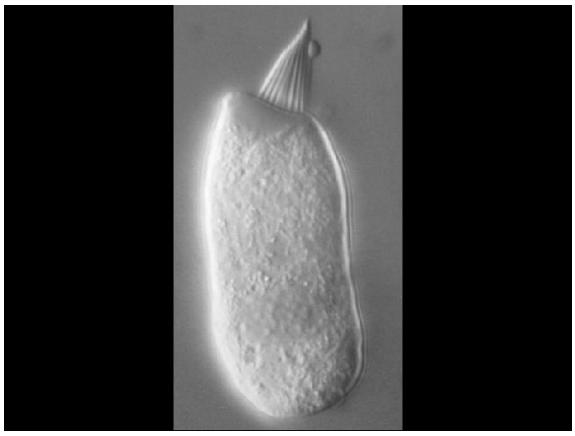

Figure 3: Isolated outer hair cell. This picture was taken from Hudspeth (1985). At the top of the hair cell, which is about 30 um long, is a hair bundle that is about 5 um wide. It is the hair bundle that moves in response to basilar membrane motion, and this motion causes motion-sensitive ion channels on the hair cell to open.

The motion of the basilar membrane causes the outer hair cells to move. In response to this motion, the outer hair cells generate an active response that produces an SF OAE, which moves either through compression of the fluid as proposed by (Ren, 2004) or as a backward-propagating traveling wave along the basilar membrane (Shera & Guinan, 2003; Shera & Zweig, 1993; Zweig & Shera, 1995). This backward traveling wave (or compression wave) vibrates the oval window which generates a pressure change within the ear canal that can be measured using a sensitive microphone. This pressure change due to the basilar membrane motion and active hair cell motion is what is called the SF OAE.

The tonotopic regions along the basilar membrane are targets for different audio frequencies. Regions closer to the base of the basilar membrane are tonotopic targets for high frequencies. As pressure waves move apically along the basilar membrane, the regions are targets for lower frequency audio signals. So if the forward propagating traveling wave peaks closer to the base of the basilar membrane, then the subject would identify that sound as having a higher frequency. Likewise, if the forward propagating traveling wave peaks closer to the apex, the subject would identify that sound as having a lower frequency.

OAEs can be evoked using different types of auditory stimuli. The different audio stimuli target responses from hair cells in different regions along the basilar membrane. There are three basic types of evoked OAEs: (1) transient evoked (TE OAEs), (2) distortion product (DP OAEs) and finally (3) stimulus frequency (SF OAEs), the mechanism of which was discussed earlier in this section. While a brief overview for methods of evoking each of these types of OAEs is provided, this work is primarily concerned with SFOAEs.

1.5.1. Transient Evoked Otoacoustic Emisssions (TE OAEs)

TE OAEs, first measured by Kemp (1978) are evoked by presenting a click audio stimulus to the ear and then recording the response in the ear canal. The response is typically measured within a window of time after which the residual stimulus reflection artifact will have subsided (2-3 ms) and extending to 20 ms after the stimulus onset. A click is a spectrally broadband stimulus, and so the OAE evoked is believed to be a response from many locations along the basilar membrane. The advantage of using clicks to evoke OAEs is that the stimulus is short and so the window of analysis is not corrupted by stimulus artifact. The disadvantage of TE OAEs is that the stimulus is broadband, which may produce complex interactions along the cochlea such as distortion products, making it difficult to interpret the response. Also, by windowing out the first 2-3 ms, it is impossible to measure high frequency OAEs. A further disadvantage to using clicks in this dissertation research is that we are studying OAEs as modulated by MOC activation. MOC has been shown to be more easily elicited by broadband stimuli including clicks (Guinan Jr. J. , 2006). When studying the effect of the MOC on OAEs, it is important to choose auditory stimuli that can independently evoke an OAE without simultaneously activating the MOC.

1.5.2. Distortion Product Otoacoustic Emissions (DP OAEs)

DP OAEs are evoked by presenting two tones simultaneously to the ear canal. The two tones are at different frequencies (f1 and f2) where f2 is higher in frequency than f1 and the frequency ration of f2/f1 is typically 1.22. This frequency ratio has been found to be the optimal choice for eliciting the most robust OAE response within a clinical setting (Gorga, Neely, Ohlrich, Hoover, Redner, & Peters, 1997). Because the two tones differ in frequency, they target different tonotopic locations along the basilar membrane – f2 closer to the base and f1 more apical. The traveling wave generated by the two tones have the largest interaction at the f2 location due to the rapid deterioration of the f2 traveling wave apical to the f2 characteristic frequency location. Therefore, it is believed that the DP OAE originates as mechanical distortion near f2. This distortion includes the 2f1-f2 distortion product and many others. The 2f1-f2 and other lower band distortion products travel bi-directionally along the basilar membrane. The 2f1-f2 distortion product reaches its tonotopic location, which is more apical than either f1 or f2, where outer hair cells generate a coherent linear reflection (Talmadge, Long, Tubis, & Dhar, 1999). While there has been research done on the effects of MOC activation on DP OAEs (Abdala, Mishra, & Williams, 2009; Purcell, Butler, Saunders, & Allen, 2008; Atcherson, Martin, & Lintvedt, 2008; Sun, 2007), we chose not to use DP OAEs in our experiments because of the increased complexity and interpretation of the generator mechanism. Specifically, the DP OAE is comprised of both a linear coherent reflection component at the 2f1-f2 location on the basilar membrane and the nonlinear distortion component near the f2 location of the overlap region of the basilar membrane and this dual generator of the response complicates the interpretation of the results.

1.5.3. Stimulus frequency OAEs (SF OAEs)

SF OAEs are also sounds generated by the cochlea in response to audio stimuli presented to the ear canal. However, an SF OAE response is a response from a specific point along the

basilar membrane that corresponds with a stimulus probe frequency. This response is believed to be a result of a linear coherent reflection generation mechanism, or phase-sensitive reflections occurring along the basilar membrane (Shera & Guinan, 2003; Shera & Zweig, 1993; Zweig & Shera, 1995). Unlike DP OAEs, the nonlinear distortion component within the response is discarded and only the response at the probe frequency is measured and analyzed. There are three ways of evoking an SF OAE; (1) nonlinear compression, (2) spectral smoothing, and (3) two-tone suppression (Kalluri & Shera, 2007). The nonlinear compression method uses the fact that SF OAE amplitudes compress at higher stimulus intensities and the amplitude of the SF OAE is estimated using this compression phenomena. The spectral smoothing method utilizes the fact that the stimulus and the SF OAE emission have different latencies associated with their generation. By convolving the stimulus ear canal pressure spectrum with a smoothing function, the SF OAE can be estimated. Finally, the suppression method relies on the presentation of two tones to the ear; a probe tone that elicits the SF OAE, and a suppressor tone that is presented at a high enough level that it is assumed to fully suppress the amplitude of the evoked OAE. Kallure & Shera (2007) showed that these three methods of measuring SF OAEs were nearly equivalent. In the work presented in this dissertation, the suppression method was used to measure SF OAEs.

When using the suppression method of SF OAE measurement, SF OAEs can be evoked by presenting a sinusoidal tone stimulus to the ear and recording the phase variations in the pressure response within the ear canal. Kemp & Chum showed (1980) that SF OAEs can also be recorded using a sine tone presented at two different levels or by presenting two sine tones very close in frequency. The latter two paradigms rely on nonlinear cochlear outer hair cell transduction to measure the SF OAE as the difference between the two-tone response and the response to each tone in the pair presented alone. This is explained in more detail below. Because tones target a narrower tonotopic region along the basilar membrane and because for low

to moderate level stimuli, there is less complexity regarding their generation mechanism as compared with both TE OAEs and DP OAEs.

An SF OAE cannot easily be measured directly because the sound pressure measured in the ear canal in response to an auditory stimulus signal consists of the stimulus signal plus the OAE as shown in Figure 4.

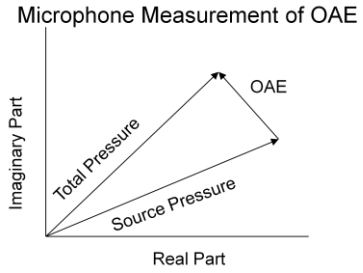

Figure 4: An OAE cannot easily be measured directly. The sound pressure measured in the ear canal is a summation of both the audio stimuli and the OAE response to the stimuli.

Keefe showed that an SF OAE can be approximated using a 3-interval nonlinear residual technique (Keefe, 1998; Keefe & Ling, 1998). Using a 2-tone suppression paradigm with a suppressor that is lower in frequency compared with the probe, an SF OAE is a measure of the cochlear suppression of the basilar membrane response to a probe tone (s_2) by a suppressor tone (s_1). The frequency of the suppressor tone (f_1) is chosen to be spectrally close (within 3-5%) to the frequency of the probe tone (f_2). Tones s_1 and s_2 are presented independently and the cochlear responses to these tones (p_1 and p_2, respectively) are measured using a sensitive microphone. Next, tones s_1 and s_2 are presented simultaneously (f_{12}). The SF OAE is the difference between the microphone measurement P_{12} and P_1+P_2 as shown in Figure 5

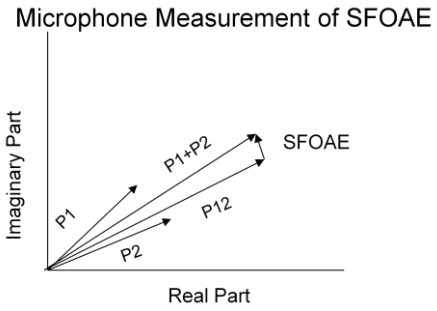

Figure 5: Measuring an SF OAE using two-tone suppression where P1 is the microphone measurement to the suppressor, P2 is the microphone measurement to a probe tone, and P12 is the microphone measurement of the probe and suppressor presented simultaneously.

The SF OAE is the nonlinear residual difference between (P_1+P_2) and P_{12} as calculated using Equation 1.

Equation 1 $\mathbf{P_d = P_1+P_2-P_{12}}$

This nonlinear residual (Pd) is a measurement of the amount that the suppressor tone has caused a reduction in the cochlear response to the probe tone when the probe and suppressor are presented simultaneously. When the suppressor tone is large enough in level, it causes a complete suppression of the cochlear response to the probe tone. When complete suppression occurs, the nonlinear residual difference represents the cochlear response to the probe tone or the OAE.

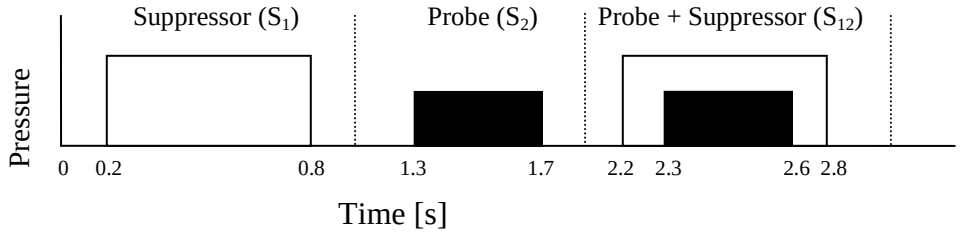

Figure 6: SF OAE stimulus paradigm using the double-evoked method (Keefe, 1998; Keefe & Ling, 1998). The white box represents the suppressor tone and the black box represents the probe tone. Notice that the suppressor is presented first (S_1), followed by the probe (S_2), and lastly the probe and suppressor are presented simultaneously (S_{12}).

The SF OAE stimulus paradigm is shown in Figure 6. The suppressor is presented first, followed by the probe stimulus, and then the probe + suppressor is presented. The SF OAE residual, is

calculated using Equation 1 where P_1, P_2, and P_{12} are the total pressure measured in the ear in response to stimulus S_1, S_2, and S_{12}, respectively.

1.6. Background on efferent inhibition of OAEs

There are two efferent pathways known to inhibit OAEs; the middle ear muscle (MEM) reflex and the medial olivocochlear (MOC) reflex (Guinan Jr. J. , 2006) . Both of these reflexes are activated by similar types of audio stimuli, which can be confounding when attempting to determine which efferent reflex caused a recorded response. Activation of hair cells along the basilar membrane excites synapses connected to the auditory nerve. Action potentials travel along the afferent auditory nerve up to the lower brainstem where, depending on the auditory stimuli, they activate the medial olivocochlear (MOC) nerve bundle and/or the middle ear muscle.

1.6.1. Middle ear muscle (MEM) reflex

The MEM reflex is activated typically by lower frequency, higher energy sound (Goodman & Keefe, 2006). An MEM reflex may be evoked by either ipsilateral or contralateral sound. The neuronal pathways for this reflex are shown in Figure 7.

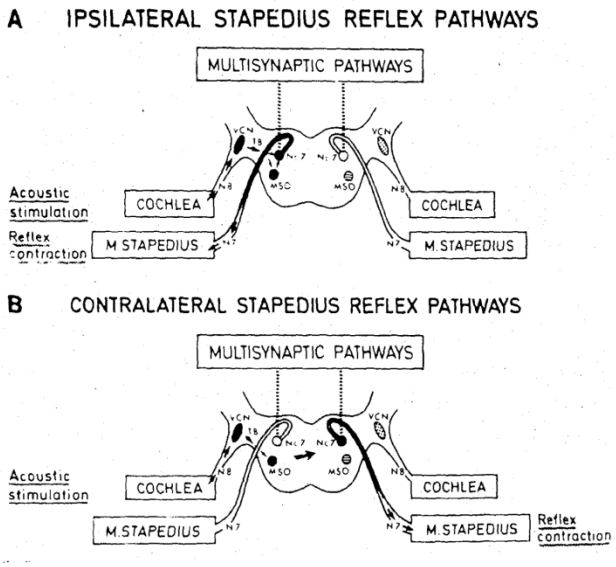

Figure 7 Middle ear muscle pathway (Katz, 2001).

There are four nerve bundles that make up the ipsilateral MEM reflex pathway. Sound enters the cochlea and activates the auditory nerve which transmits to the ventral cochlear nucleus (VCN). The axons within the VCN activate the facial motor nucleus which enervates the stapedius muscle, causing it to flex. However some of the VCN axons first activate the ipsilateral medial superior olivary complex (MSO), which then activates the facial motor nucleus, causing the stapedius to flex. The contralateral pathway always travels through four neuron bundles: the auditory nerve, the VCN, the medial superior olivary complex, and finally the contralateral facial motor nucleus which enervates the contralateral stapedius muscle (Katz, 2001). The flexing of the stapedius muscle increases the acoustical impedance of the ear which reduces the amount of sound transmitted to the cochlea for generating OAEs and increases the stimulus reflection artifact measured in the ear canal. Since the change in acoustical impedance due to the MEMR is so much larger than any OAE being measured, the activation of the MEMR overpowers any response that could be due to changes in the OAE response (Borg, 1974; Katz, 2001).

1.6.2. Medial Olivocochlear Efferent

The medial olivocochlear reflex pathway is similar to the MEM pathway. Sounds such as noise and clicks enter through the cochlea and activate the auditory nerve, which activates the VCN. The VCN activates the medial olivocochlear efferent which innervates the outer hair cells and reduces their motility.

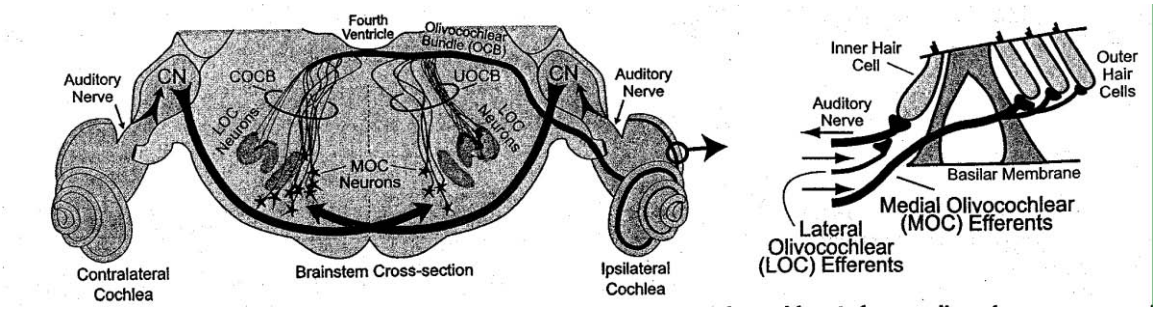

Figure 8: Medial olivocochlear efferent pathway (Guinan Jr. J. , 2006).

Because the pathway of the MEM and MOC reflexes are very similar, this complicates the interpretation of results from tests which are targeted towards evoking one or the other reflex (Guinan Jr. J. , 2006).

1.7. Distinguishing between MEMR and MOC inhibition effects

In the experiments described in this dissertation, broadband noise stimuli were used to elicit the MOC. In our evaluation testing, subjects were screened to determine at what level the MOC elicitor began to evoke the MEM so that we could avoid testing near those levels for the purpose of making MOC inhibition measurements. We used a procedure whereby the SF OAE was elicited by presenting a low-frequency (602 Hz) moderate level (55 dB SPL) tone to the subject's ear while the MOC noise elictor level was presented simultaneously; the noise elicitor was presented ipsilaterally, contralaterally, and bilaterally using increasing levels from 55 up to 85 dB SPL. Changes in pressure in the ear canal (both amplitude and phase) were monitored. The changes caused by the elicitor were monitored during elicitation (for contralateral elicitors) or within a post-elicitor window 40 ms after the elicitor had turned off (for ipsilateral and bilateral elicitors). If a change in amplitude or phase was observed at a given elicitor level, that elicitor level was considered the MEMR threshold. For experiments in which the MOC alone was elicited, elicitor levels were used that were at least 5 dB below this MEMR threshold for each subject.

1.8. Auditory model

Results from this dissertation research can be used to extend current auditory models so that physiologic factors including blood glucose, attention, and aging are incorporated into the models. There are several prevailing auditory models that functionally describe certain key aspects of auditory signal processing. All models consist of separate stages which describe specific physical regions of the auditory processing signal path as an auditory signal enters the

outer ear and proceeds to the cochlea and finally up to the auditory cortex. A higher level general model is shown in Figure 9.

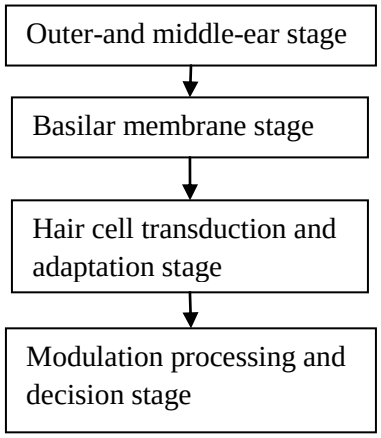

Figure 9: General cochlear model (Jepsen, Ewert, & Dau, 2008).

We will primarily be discussing the basilar membrane stage model and extending it to include blood glucose and attention impacts on the efferent pathway as well as aging effects on the cochlear tuning and gain within the basilar membrane.

1.9. Current auditory models

Historically, the frequency decomposition performed by the basilar membrane has been modeled as a transmission line as shown in Figure 10.

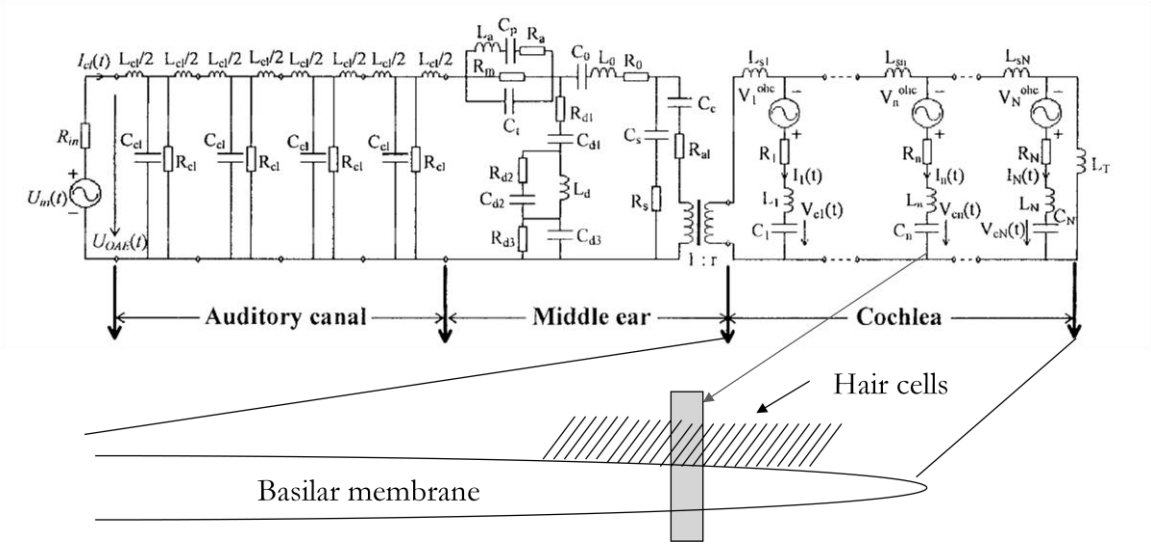

Figure 10 Transmission line model of the auditory periphery (Giguere & Woodland, 1994).

A more recent model called the dual resonance nonlinear model (DRNL) was developed by Meddis et al. (2001) and is used by some researchers instead of the transmission line model because it is computationally more efficient and because the DRNL estimates of basilar membrane filter bandwidths more closely resembled the bandwidths in the cochlea (Jepsen, Ewert, & Dau, 2008). The DRNL model is shown in Figure 11.

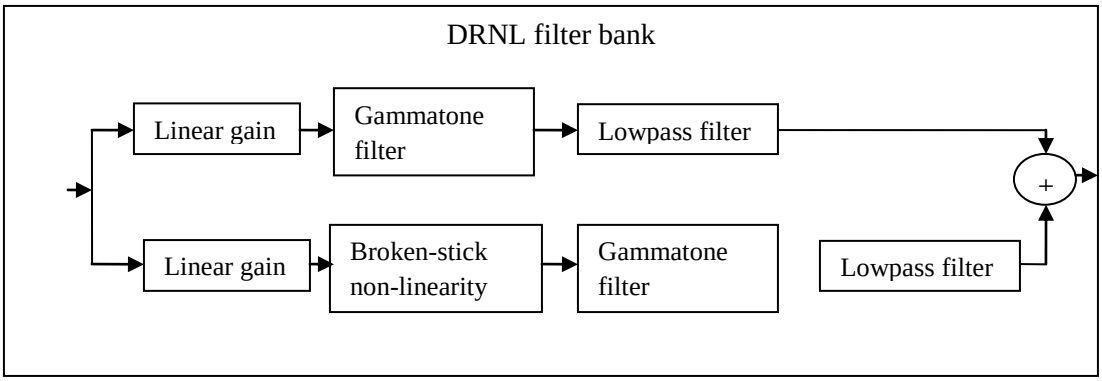

Figure 11: Basilar membrane model proposed by Meddis et al. (2001).

1.10. Extending auditory model to include MOC effects

The Meddis model in Figure 11 was extended by Ferry and Meddis (2007) to include a representation of the attenuation of basilar membrane motion caused by activation of medial

olivocochlear efferent. The extension of the DRNL filter bank is shown in Figure 12 and simply includes a 1-parameter attenuation block. The parameter of attenuation is dependent on the level of MOC elicitation.

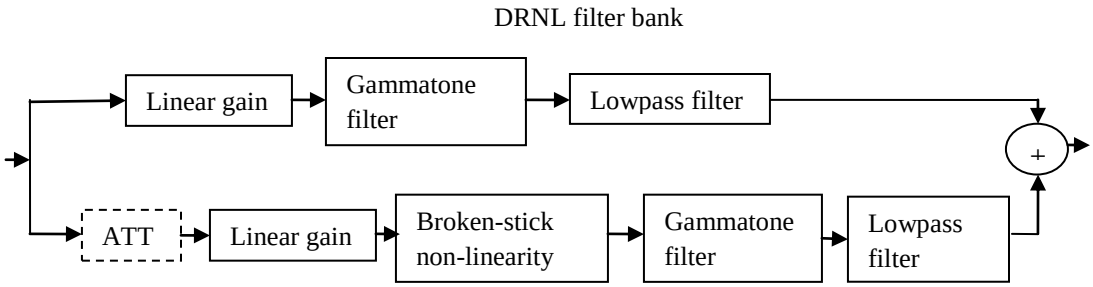

Figure 12: Extension of DRNL filter bank to include attenuation of basilar membrane motion due to MOC activation (Ferry & Meddis, 2007). The extension is shown in dashed lines.

The Ferry and Meddis extension of the model is helpful in that it begins to describe the effects of MOC activation on attenuating basilar membrane motion. However, the model has several critical shortcomings. The primary shortcoming is that it fails to model the time-varying nature of the MOC including the rise-time and latency of the MOC activation. It also fails to model the types of stimuli that are known to activate the MOC – broadband noise and clicks. Instead, the model assumes that the MOC elicitor is stimulated independent of the auditory stimuli. This type of model is effective for animal experiments in which the MOC is elicited by an electrode connected to individual MOC nerve fibers. In this case, the exact amplitude of the MOC elicitation is known and independent of the auditory stimuli being presented to the ear canal. However, when the auditory stimuli itself initiates activation of the MOC, it is imperative that the model accurately describe how the MOC activation is caused by specific stimuli. This is the case for example, when broadband noise or clicks are combined with tones. The noise and clicks will activate the MOC, and thereby attenuate the basilar membrane motion. The Ferry and Meddis

model does not account for complex auditory stimuli, of which some elicit MOC activation (such as higher level broadband noise and clicks) while some do not (such as lower-level tones).

1.11. Extending model to show physiologic impacts on MOC and OAE

In this dissertation work, the Ferry and Meddis model has been extended to include (1) blood glucose impacts on the MOC magnitude and SF OAE amplitude, (2) attention impacts on the MOC magnitude, (3) aging impacts on the SF OAE amplitude and (4) timing components of MOC activation.

DRNL filter bank with extensions including physiologic effects on MOC and cochlear gain

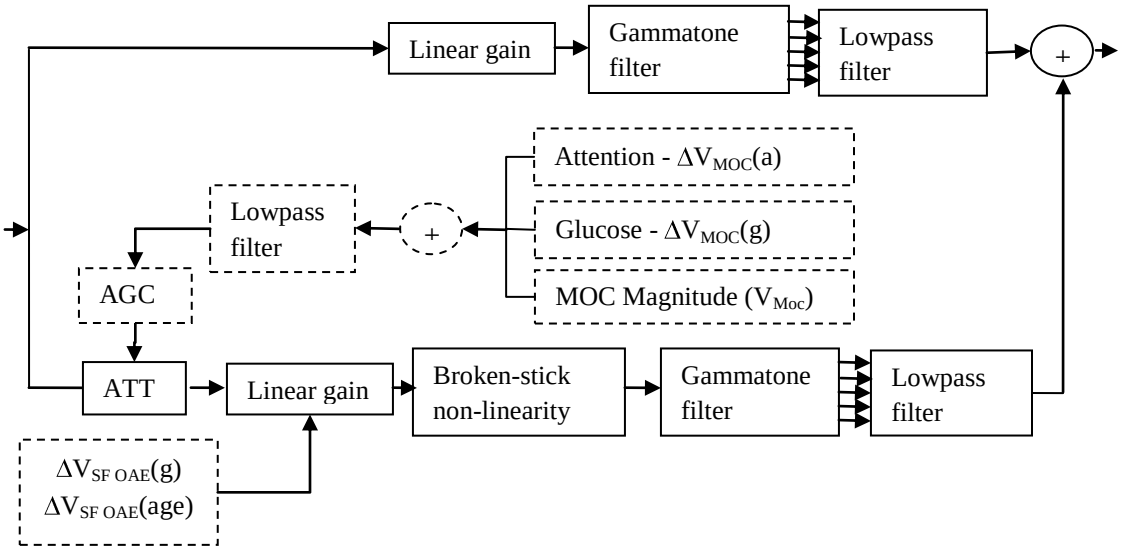

Figure 13: Model extensions of basilar membrane to include glucose and attention impacts on MOC. The model also shows impacts of aging on the gain and latency.

A low-pass filter, modeled as an RC circuit, is used to model rise-time and latency characteristics of the MOC activation.

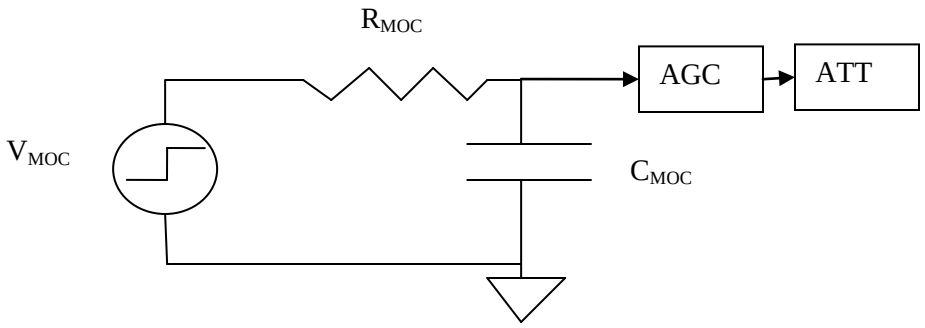

Figure 14: MOC activation modeled as a low-pass filter circuit. R and C components represent lumped nerve properties from the MOC. R_{MOC} represents the lumped ion channel resistance while C_{MOC} represents lumped ion channel capacitance

When modeled as such, the time-varying aspect of the MOC can be represented as either a rising or falling exponential depending on if the MOC is turning on or off, respectively. For example, when the MOC is turned on, it can be modeled using Equation 2.

Equation 2

$$V(t) = V_{MOC}(1 - e^{-(t-L)/\tau_{MOC}})$$
$$\tau_{MOC} = R_{MOC}C_{MOC}$$
$$V_{MOC} = \begin{cases} MOC\_Amplitude : t \geq L \\ 0 : t < L \end{cases}$$

The extended MOC activation model includes blood glucose (g) and attention effects (a) on MOC complex magnitude (V), latency (L) and rise-time (τ).

Equation 3

$$V_{MOC\_pred}(t,g,a) = (V_{MOC} + \Delta V(g) + \Delta V(a))(1 - e^{-(L_{MOC}+\Delta L(g)+\Delta L(a))/(\tau_{MOC}+\Delta\tau(g)+\Delta\tau(a))})$$

In chapter 2, we show that hyperglycemia results in higher MOC complex magnitude (ΔV(g)) but that glucose did not affect MOC rise-time or latency. Likewise, in chapter 3, we show that active attention to a task results in a larger MOC complex magnitude as compared with passive listening (whereby no attention is being used) in grouped data. MOC rise-time and latency are not affected by active vs. passive attention to a task. From these results, Equation 3 can be simplified to only include amplitude effects of glucose and attention on the MOC as is shown below in Equation 4.

Equation 4 $$V_{MOC\_pred}(t,g,a) = \left(V_{MOC} + \Delta V(g) + \Delta V(a)\right)\left(1 - e^{-\left(\frac{t - L_{MOC}}{\tau_{MOC}}\right)}\right)$$

The SF OAE onset/offset timing characteristics can also be modeled as a low-pass filter as shown in the Ferry and Meddis model in Figure 13 at the end of the nonlinear (lower branch) section of the DRNL model. In chapter 4, aging effects on SF OAE amplitude and latency are investigated and the extension to the low-pass filter is shown in Figure 13. To model aging effects on SF OAE amplitude and latency, Equation 5 below is used.

Equation 5 $$V_{SFOAE\_Pred}(t,age) = \left(V_{SFOAE} + \Delta V(age)\right)\left(1 - e^{-\left(\frac{t - L_{SFOAE} + \Delta L(age)}{\tau_{SFOAE}}\right)}\right)$$

In Equation 5, $V_{SF\,OAE}$ is the amplitude of the SF OAE for young people and $\Delta V(age)$ is the component of the SF OAE amplitude that changes due to aging. Likewise, $L_{SF\,OAE}$ is the latency of the SF OAE for young people and $\Delta L_{SF\,OAE}$ is the change in SF OAE latency due to aging. Finally, $\tau_{SF\,OAE}$ is the rise-time of the SF OAE. We did not expect aging effects to impact the rise-time of the SF OAE, therefore there is not a variable representing the change in rise-time in Equation 5 showing changes in this parameter due to aging.

1.12. Summary

In this introduction chapter, we have presented the primary research objectives of this dissertation. Specifically, our research objectives are to (1) demonstrate how the auditory system changes with blood sugar and investigate methods for using OAE and MOC metrics to predict blood sugar, (2) evaluate whether the MOC is a response that is cortically controlled and therefore influenced by auditory attention, and finally (3) investigate how aging impacts the auditory system independent of hearing impairment. An overview of each of these three research areas has been presented and the relevance of the current studies to the field has been given. We have discussed our findings and summarized the major contributions of this research. We also presented a background on OAEs, the MOC efferent system, middle ear muscle reflexes, and

current auditory models. And we have discussed how findings from this research may ultimately fit into current auditory models. In the next three chapters, results from each of these three studies are discussed independently. And in the concluding chapter, we discuss how the findings can be considered as a whole.

Chapter 2

2. Inferring blood glucose from OAEs and efferent inhibition of OAEs

The primary objective of this dissertation research is to explore the relationship between blood glucose, OAEs, and efferent inhibition of OAEs and to utilize this relationship to create a predictive model that could ultimately be clinically relevant as a noninvasive glucose monitoring method. In this chapter, an overview is provided on the state-of-the art invasive and noninvasive blood glucose monitoring. A background is presented on the two proposed biological mechanisms that explain why blood glucose impacts OAEs and MOC inhibition. Prior work is presented on experiments done on humans and animals showing OAE changes with blood sugar as well as more general experiments showing how nerve amplitude and latencies have been shown to vary with blood glucose. The methodology is described with regards to how OAEs were evoked and how MOC inhibition was elicited and measured. Statistical models are described and a new method for estimating hyperglycemia called Hyperglycemia Risk Analysis is presented. Finally, results are presented that show which OAE and MOC inhibition metrics demonstrated the strongest correlation with glucose and how these metrics were used effectively in a cross-validated model prediction scenario.

2.1. Background

People with diabetes have blood glucose that can vary substantially during the day and also when they are asleep. The standard for diabetes care is for patients to regularly monitor the blood glucose using home capillary blood glucose monitoring systems (American Diabetes Association, 2001; Hirsch, Farkas-Hirsch, & Skyler, 1990). If continuous monitoring is not an option, the American Diabetes Association has recommended that patients with type 1 diabetes check three to four times per day and people with type 2 diabetes check at least twice each day (American Diabetes Association, 2001). Consequences of failure to regularly monitor can be

severe during hypoglycemia (low blood sugar) whereby patients could suffer from a seizure and die if not treated immediately. And there are long-term consequences from high sugar levels that can lead to peripheral neuropathy, nephropathy, ophthalmic abnormalities, organ damage, and cardiovascular disease (Killilea, 2002). However, despite the evidence showing the harm in failing to monitor, many patients with diabetes fail to routinely check their sugar levels (Burge M. R., 2001). Researchers have been searching for many years without success for ways to measure blood glucose noninvasively in diabetic subjects (Tura, Maran, & Pacini, 2007). Noninvasive glucose monitoring methods have historically fallen under the category of either optical methods or electrical methods (Tura, 2008). Optical methods utilize near infrared light to noninvasively obtain spectrally relevant signatures that are known to correlate with glucose levels (Burmeister & Arnold, 1999; Saptari & Youcef-Toumi, 2004; Saptari & Youcef-Toumi, 2005; Liu & Arnold, 2009). Electric methods have used radio wave impedance spectroscopy and alternatively iontophoresis whereby electrical currents are applied to the surface of the skin to extract tiny samples of blood for analysis (McGarraugh, 2009). Electrical and optical approaches to noninvasive blood glucose monitoring pose problems, including inaccuracy, skin irritation, and calibration problems (Sieg, 2005; Zheng, 2000). Work from this dissertation is the first to propose a noninvasive glucose monitoring method using the OAE and efferent inhibition of the OAE as the measurement metric.

2.1.1. Background on how glucose perfuses the cochlea

There are three chambers within the cochlea, the scala vestibuli, the scala timpani, and the scala media. The fluid within these chambers is perilymph (scala vestibuli and scala tympani) and endolymph (scala media). There is evidence that there is a blood-labyrinth barrier for glucose between the perilymph in the cochlea and the blood stream. This was demonstrated by Juhn and Youngs (1976) who observed that while glucose levels in perilymph parallel those in blood, there is a delay of about an hour between the time of maximum concentration of glucose in

the perilymph and its maximum concentration in blood. Endolymph is further insulated from blood glucose variation. Silverstein (1971) demonstrated that when cats were intravenously infused with 50% glucose solution, the endolymph glucose levels were unchanged while the perilymph glucose levels rose. The cationic makeup of the perilymph and endolymph is directly responsible for maintaining the endolymphatic potential which is required for auditory functionality, and this directly impacts cochlear microphonics and OAEs. Studies in microphonics have shown that variations in blood glucose levels affect cochlear microphonic amplitude, but only after a significant delay of 2-3 hours (Mendelsohn & Roderique, 1972; Juhn, Rybak, & Fowlks, 1982). The delay is most likely due to the labyrinthine barrier between blood and the endolymph and perilymph fluids which maintains a more constant glucose supply than what is in the blood stream. A similar delay might be predicted for OAEs, since they are caused by outer hair cell motility which is linked to the outer hair cell receptor potential.

2.1.2. Mechanisms for glucose impacts on OAE and MOC inhibition

There are two mechanisms that could explain and help predict how blood glucose affects OAEs and MOC inhibition of OAEs. The first mechanism is based on the concept that glucose is an energy source for the cochlea and this mechanism provides an explanation for how blood glucose levels could impact OAE amplitudes. The second mechanism is based on the concept that ATP, produced through the breakdown of glucose through glycolysis, is a neurotransmitter that regulates the motility of outer hair cells. This second mechanism provides an explanation for how blood glucose levels impact the MOC inhibition of OAEs. Both of these mechanisms are further discussed below.

2.1.2.1. Mechanism for glucose impact on endocochlear potential

Glucose is the primary energy source for the cochlea (Kambayashi, Kobayashi, Demott, Marcus, Thalmann, & Thalmann, 1982; Kambayashi, Kobayashi, Marcus, DeMott, Thalmann, &

Thalmann, 1982) and disruption of this energy source is expected to significantly change the function of the cochlea (Mendelsohn & Roderique, 1972; Wing, 1959; Koide, 1958). Energy is required to maintain the large voltage gradient between the outer hair cells and the endolymph, fluid in which outer hair transduction channels are bathed. The endolymph has a positive potential of 80-90 mV as first demonstrated by Tasaki and Spiropolis (1959). The outer hair cells have a potential of approximately -50 mV, making the endolymphatic potential difference the largest in the body. The endolymph has a high potassium ion (K+) and a low sodium ion (Na+) concentration (Fernandez, 1967; Mendelsohn & Konishi, 1969; Smith, Lowry, & Wu, 1954). This large concentration gradient requires an active transport of Na+ out of the endolymph and K+ into the endolymph; this active transport mechanism occurs in the stria vascularis which lines the lateral wall of the scala media (Kuijpers, 1969; Prazma, 1969). Energy for this active transport mechanism comes in the form of glucose, which has been converted into ATP through the breakdown of glucose.

Mendelson and Roderique (1972) showed that when you deprive the endolymph of glucose in the guinea pig, the high K+/Na+ gradient between endolymph and hair cell is reduced. Wing (1959), Koide (1958), and more recently Angelie et al. (2009) reported that hypoglycemia leads to a reduction of the endocochlear potential and a related decrease in cochlear microphonics. When the endocochlear potential is reduced experimentally using furosemide injection, distortion product OAEs are reduced in amplitude (Mills, Norton, & Rubel, 1993). This reduction in DP OAEs during hypoglycemia was verified by Zuma e Maia et al. (2006; 2008) who found that when sheep were given a bolus of insulin, thereby lowering their blood sugar levels, distortion product OAEs declined in amplitude. The reduction in amplitude of the DP OAE occurred primarily in higher frequency DP OAEs and the decrease occurred about 60 minutes after induction of hypoglycemia. The timing of the decrease in DP OAE amplitude during hyperglycemia agrees with work by Mendelsohn and Juhn as they expected a delay due to

the endolymph / blood labyrinthine barrier (Mendelsohn & Roderique, 1972; Juhn, Rybak, & Fowlks, 1982). However, not all results have been consistent. There has also been work showing that OAEs do not change with blood sugar (Sasso, et al., 1999). And there was a study showing that OAE amplitude is inversely proportional to glucose level. Specifically, Suckfull et al. (1999) showed that OAE amplitudes in the rabbit decreased during cochlear perfusions with high glucose concentrations but did not change during perfusions with lower glucose concentrations. None of the groups investigated SF OAEs and timing metrics were not considered. The conflicting prior results merited a further look into how blood glucose levels impact OAE metrics. Our hypothesis that OAE amplitude would increase during hyperglycemia is based on the majority of the prior work indicating that hypoglycemia will lead to a reduction in EP and smaller cochlear microphonics and OAEs.

2.1.2.2. Mechanism for blood glucose impact on MOC

In the previous section, a mechanism was described that hypothesizes that higher OAE amplitudes will be observed during hyperglycemia. However, there is a different mechanism that is described in this section that hypothesizes that inhibition of OAEs will be larger during hyperglycemia; but this mechanism is dependent on the simultaneous activation of the MOC efferent pathway. This mechanism is based on the concept that ATP has been found to be an important neurotransmitter in the hair cell and in other supporting cells within the organ of Corti (Bobbin & Thompson, 1978). In addition to being used for energy within the cochlea, it is known that ATP is an important neurotransmitter and that there are ion channel receptors (called P2X) localized to outer hair cell base that are sensitive to ATP and also to ATP antagonists (Yu & Hong-Bo, 2008; Kujawa, Fallon, & Bobbin, 1994b; Bobbin & Thompson, 1978; Munoz & Thorne, 1995; Munoz, Thorne, & Housley, 1999; Sueta, Paki, Everett, & Robertson, 2003). Kujawa et al. (1994a) showed that DP OAE amplitudes and also auditory nerve compound action potentials were significantly reduced when ATP and ATP analogues were injected into the

perilymph. Kujawa et al. also found (1994b) that these ATP-dependent effects could be reversed when ATP ion channel antagonists were applied to the perilymph, thereby confirming that ATP-sensitive ion channels exist in the outer hair cells. Skellet et al. (1997) confirmed the effect of ATP on DP OAE amplitudes by showing that application of suramin, an ATP antagonist, to perilymph caused larger DP OAE amplitudes when DP OAEs were being evoked continuously over long time intervals. Several groups have shown that these ATP-ion channel effects are dependent on the presence of calcium ions in the extracellular fluid surrounding outer hair cells (Ashmore & Ohmori, 1990; Nakagawa, Akaike, Kimitsuki, Komune, & Arima, 1990; Dulon, Mollard, & Aran, 1991; Housley, et al., 1999; Yu & Hong-Bo, 2008). These groups have shown that application of ATP to an outer hair cell leads to inward, depolarizing currents and uptake of calcium ions into the outer hair cell. This depolarization of the hair cell can have the result of reducing the gain of the cochlear amplifier and thereby reducing OAE amplitudes (Yu & Hong-Bo, 2008). It has been proposed that acetylcholine (Ach) is what is responsible for the release of the extracellular calcium ions (Frolenkov, Mammano, Belyantseva, Coling, & Kachar, 2000; Blanchet, Erostegui, Sugasawa, & Dulon, 1996; Evans, 1996). And the MOC has been shown to release Ach (Elgoyhen, Vetter, Katz, Rothlin, Heinemann, & Boulter, 2001; Guinan Jr. J. J., 2010). Therefore, it is reasonable to make the hypothesis that ATP, acting as a neurotransmitter, along with calcium ions released by the MOC, causes an increase in depolarization of outer hair cells and thereby generates a reduction in OAE amplitudes. The hypothesis is therefore that hyperglycemia will lead to increased inhibition of the OAE amplitude when regulated by the activation of the MOC.

Glucose impact on other nerves: Since the MOC is a nerve bundle, it makes sense to consider other research that has investigated nerve amplitudes and transmission latencies as they vary with blood sugar levels. Peripheral nerve conduction speeds have been shown to vary immediately with changes in blood glucose levels (Sindrup, Ejlertsen, Gjessing, Froland, & Sindrup, 1988).

Glucose has also been shown to affect cortical auditory processing (McCrimmon, Deary, & Frier, 1997) and auditory nerve pathways, including evoked responses and axonal transmission latencies during the hyperglycemic state (Rayner, et al., 1999; Russo, et al., 1999; Sindrup, Ejlertsen, Gjessing, Froland, & Sindrup, 1988). Studies of the auditory brainstem response (ABR) have shown central conduction time delays in diabetic subjects (Bayazit, Bekir, Gungor, Kepekci, Mumbuc, & Kanlikama, 2000; Bayazit, Yilmaz, Kepekci, Mumbuc, & Kanlikama, 2000; Lisowska, Namyslowski, Morawski, & Strojek, 2001; Nakamura, Takahashi, Kitaguti, Imaoka, Kono, & Tarui, 1991; Sasso, et al., 1999), changes during insulin-induced hypoglycemia (Deutsch, Sohmer, Weidenfeld, Zelig, & Chowers, 1983; Ziegler, Hubinger, & Gries, 1991), and changes during glucose clamp induced hyperglycemia conditions (Sasso, et al., 1999). De Feo et al. (1988) demonstrated an inverse relationship between ABR wave latencies and plasma glucose. While the OAE is not itself a nerve response like the ABR, OAE amplitudes are diminished by activation of MOC efferent neural projections to the cochlea from the brainstem. The fact that there is prior research showing impacts of hyperglycemia and hypoglycemia on nerves provided us with further evidence for investigating the impact of blood glucose on the MOC nerve bundle.

2.2. Methods

Several experiments were done to determine whether amplitude, phase, and timing properties of OAEs and MOC inhibition waveforms correlate with blood glucose levels. A study was done on 6 diabetic subjects (5 subjects tested twice for a total of 11 trials). In these trials, lower amplitude noise elicitors were used to determine whether MOC responses, uncorrupted by MEMR, correlate with blood glucose levels. A second study was done on 12 non-diabetic subjects (6 tested twice for a total of 18 trials) using higher amplitude elicitor levels. Higher level noise elicits both the middle ear muscle and the MOC. Therefore, the experiment on the non-diabetic subjects tested whether middle ear muscle reflex effects combined with MOC effects demonstrated a correlation with blood sugar levels in subjects not suffering from diabetes. Both

experiments involved eliciting and recording the medial olivocochlear (MOC) efferent response by presenting ipsilateral, contralateral and bilateral noise stimuli to the ear while recording the response of the cochlea to a tone. During the experiments, blood glucose levels were elevated during the experiment, and MOC responses were monitored during a baseline period and then during a hyperglycemic state. Prediction of glucose using MOC amplitude and rise-time was done using a linear mixed effects model. Model accuracy was evaluated using leave-one-out cross validation.

In addition to the two studies described in this chapter, a paper is included (Jacobs, Wan, & Konrad-Martin, 2008) as an appendix to this dissertation that displays early pilot data on several diabetic and nondiabetic subjects who were tested using similar protocols to the ones described below.

2.2.1. Diabetes experiment summary

The first experiment described in this chapter was done on six diabetic subjects, five of whom returned on another day to repeat the testing to determine test-retest performance. For this experiment, we used low level MOC elicitors; broadband noise at 60-70 dB SPL. The subjects were carefully screened for middle ear muscle reflexes using the procedure described in section 1.7 and the elicitor stimuli were selected to be at least 5 dB less than levels found to elicit the MEMR in these subjects. Also in this first experiment, the attention of each subject during the glucose tolerance testing was carefully controlled; subjects were required to watch a silent movie with subtitles during the entire test.

2.2.2. Non-diabetes experiment summary

The second experiment described in this chapter was done on 12 non-diabetic subjects, 6 of whom returned on a second day for a retest. The elicitor levels for this second experiment were higher (75-80 dB SPL), and because of this both the MEMR and the MOC responses were

being elicited in all of the subjects tested. Furthermore, the attention of these subjects was not as carefully controlled during this experiment as some of the subjects watched silent movies with subtitles while others read or else were passive during the testing.

2.2.3. Subject recruitment and screening

Subjects were recruited from the Portland VA Medical Center, OHSU, and through Internet solicitations, all approved by the local IRB. Subjects were initially given a phone screening questionnaire to ensure that that they met the study criteria. The study inclusion/exclusion criteria are listed in Table 1.

Inclusion Criteria	Exclusion Criteria
• Age 18 or older For diabetic patients: • Type 1 or type 2 diabetes, diagnosed by ADA criteria (i.e. fasting plasma glucose over 126 mg/dL or 2 hour glucose over 200 mg/dl after 75 gram glucose tolerance test). • Duration of diabetes: less than 15 years. (longer durations often are associated with subclinical impairment that could confound OAE measurement results) • Hemoglobin A1C of 5.5-8%	• Hearing loss, defined as auditory threshold below 20 dB HL for frequencies under 3 kHz. • Advanced chronic kidney disease (serum creatinine of $\geq$3.0) • Hepatic dysfunction, defined as serum ALT or AST >3x the upper limit of normal or hepatic synthetic insufficiency as defined as a serum albumin of less than 3.5 g/dl • Hematocrit < 32% • Congestive heart failure NYHA class 3-4 • Active coronary artery disease as manifested by unstable angina, or a myocardial infarction or therapeutic coronary percutaneous intervention procedure (e.g. PTCA, stent placement) or CAGB within the prior 3 months. • Cerebrovascular accident within the prior 6 months • Active foot ulceration or peripheral arterial disease judged by the study physician to be severe. • Active viral infection such as HIV or hepatitis • Evidence of other active infection, including pneumonia, gastroenteritis, or urinary tract infection • Active alcohol abuse, substance abuse • Schizophrenia, bipolar disorder, or active major depressive disorder • Pregnancy • Current use of oral corticosteroids. • Contraindication to caffeine, for reasons such as cardiac arrhythmia; will not be allowed to participate in the caffeine administration portion of the study. • Contraindication to acetaminophen, for reasons such as liver damage; will not be allowed to participate in the acetaminophen administration portion of the study. For diabetic subjects: • Diabetic peripheral neuropathy as defined by loss of ability to detect light touch, position sense and vibration sense of the toes. • Visual impairment from diabetic retinopathy

Table 1: Subject inclusion / exclusion criteria.

2.2.4. Glucose tolerance test (GTT) procedures

During the glucose correlation experiment, MOC and SF OAE responses were evoked in diabetic and non-diabetic test subjects while the subjects underwent a glucose tolerance test (GTT). Subjects arrived at the clinic in the morning having fasted for at least 8 hours prior to the

test. Subjects sat in a quiet sound-proof room and had their MOC and SF OAE elicited in 10-minute presentation windows over the course of a 3 ½ - 4 hour test period. The MOC was elicited using the procedure described in Section 2.2.5. SF OAEs were evoked using a double-evoked method (Keefe, 1998; Keefe & Ling, 1998) further described below. Baseline ipsilateral, contralateral and bilateral MOC measurements and SF OAE measurements were recorded in the test subjects for 1 hour during a "baseline" period when glucose homeostasis occurred. After the baseline recording period, the subjects consumed 80 grams of cane sugar mixed with 8 ounces of water. MOC and SF OAE recordings were continued for approximately 2.5 hours after consumption of the sugar. Approximately every 10 minutes, subjects were asked to prick their finger with a sterile needle, acquire a small sample of blood, and enter it into an off-the-shelf Abbott brand blood glucose meter. These recordings were used to determine the true blood glucose level of the subject throughout the experiment so that we could determine whether SF OAE and MOC inhibition amplitude and timing metrics correlate with blood sugar.

2.2.5. Data acquisition for recording OAEs and MOC inhibition

The OAE data acquisition system consisted of a desktop PC, a Lynx2B 24-bit 6 channel sound card for presenting and recording acoustical signals on multiple channels, an Etymotic ER-2 ultra low-noise insert speaker phone, a custom built low-noise audio amplifier, and an ER10B+ sensitive insert microphone for recording the responses. The data acquisition system also required software for managing the presentation and recording of the audio waveforms. The complete data acquisition system used in this project is shown in Figure 15.

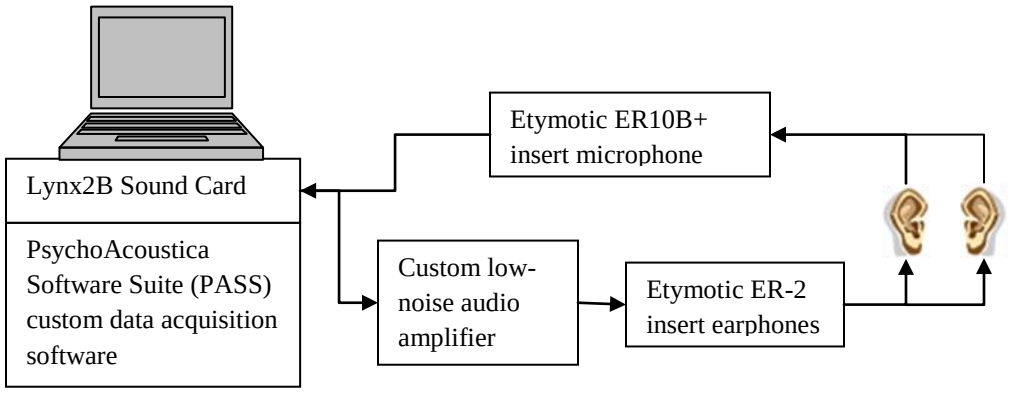

Figure 15: Custom designed OAE recording system.

The custom software, which runs on a desktop PC, was designed specifically for the studies described herein and is called PsychoAcoustica Software Suite (PASS). The PASS software is capable of executing a variety of OAE stimulus paradigms while also incorporating the ability to do simultaneous psychoacoustic testing.

Many challenges had to be overcome in the design of the data acquisition system for this project. The largest issue to be overcome was the noisy nature of the OAE recordings and the fact that everyone's ear canals are different. To help address this issue, signal processing strategies were devised and implemented as described further in 2.2.6 and 2.2.7. Furthermore, an adaptive in-ear calibration routine was designed. The in-ear calibration adjusted the levels of the audio stimuli to account for the different size ear canals. The amplitude of the stimuli was automatically adjusted to be larger in level for larger volume ear canals, and smaller in level for smaller volume ear canals.

2.2.6. Methods for measuring and analyzing MOC and MEMR

We used procedures described in (Guinan Jr. J. , 2006) to elicit and record both MOC responses and MEMR. In this procedure a 55 dB SPL audio tone was presented to the ear of the test subject for 12 seconds. The purpose of this tone was to evoke the SF OAE in the subject's

ear. During the 12-second recording period, broadband noise was presented in the ipsilateral ear, contralateral ear, and bilaterally for one second each. These one-second noise bursts have the potential to activate both the MOC and the MEMR depending on the level of the elicitor and also the individual subjects' MEMR threshold. Larger level elicitors have been shown to elicit both the MOC and the MEMR while lower level elicitors only elicit the MOC (Guinan Jr. J. , 2006).

A 1.96 second post-elicitor window was used immediately following the presentation of the elicitor signal to monitor the effect of the elicitor on the cochlear response to the tone. During the post-elicitor window, the pressure changes within the ear canal were measured and the effect that the elicitor had on the audio tone that is being continuously presented was determined. For the ipsilateral and bilateral elicitors, the post-elicitor window began 40 ms following the end of the elicitor and continued for 1.96 seconds. The reason for starting 40 ms following the end of the elicitor is to allow for time for two-tone suppression effects to subside as these affects are known to end in under 10 ms while the time constant of the MOC is on the order of hundreds of milliseconds (Guinan Jr. J. J., 1990; Guinan Jr. J. J., 1996; Tavartkiladze, Frolenkov, & Artamasov, 1996). A timing diagram for the elicitation paradigm is shown in the figure below.

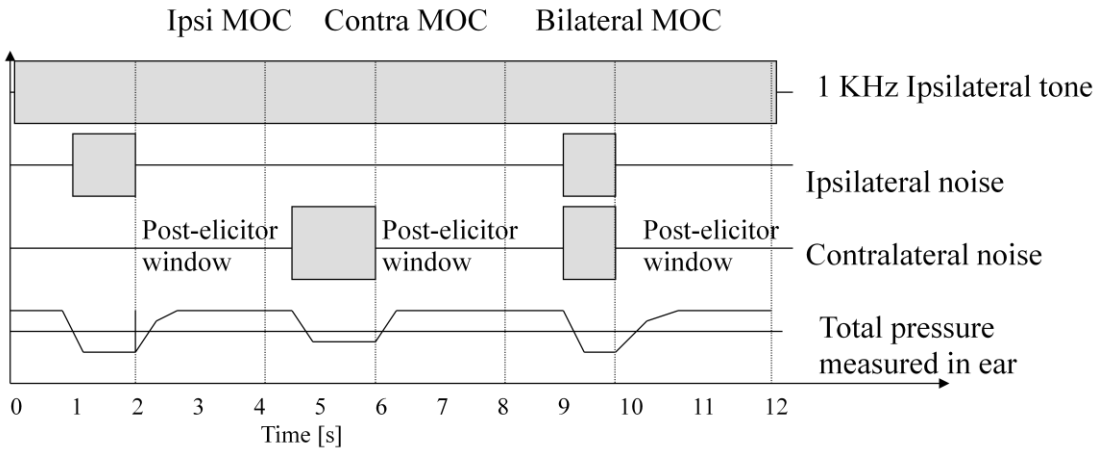

Figure 16: Timing diagram for eliciting and measuring MOC and MEMR responses. Notice that the total pressure measured in the ear declines during presentation of the elicitors. The MOC reduces the gain of the cochlear amplifier whereas the MEMR reduces the input impedance of the ear canal. Both appear to reduce the total pressure in the ear.

During the post-elicitor window, the total pressure in the ear was recorded. The presentation shown in Figure 16 was done 50 times sequentially to enable removal of outlier data and averaging of the responses. The presentation required approximately 10 minutes to complete. In between presentations, subjects were asked to prick their fingers with a sterile needle and then enter their blood into an Abbott blood glucose analyzer so that the true blood sugar could be recorded.

2.2.6.1. Signal processing for extracting MOC amplitude and timing

The activation of the MOC affects both the amplitude and phase of the OAE (Guinan Jr. J. , 2006). To estimate the MOC, a tone is presented to the ipsilateral ear to evoke the SF OAE. The total pressure is measured in the ear canal and this is called the baseline window measurement. Next, while the tone is still being presented, a noise is presented to either ipsilateral ear, the contralateral ear, or bilaterally to elicit the MOC. The amplitude and phase of the total pressure measured in the ear canal during MOC elicitation (for contralateral elicitors) or within the post-elicitor window (for ipsilateral and bilateral elicitors) is vector subtracted from the total pressure measured in the ear canal during the baseline window. Vector subtraction involves determining the real and imaginary components of the acoustical signal during both elicitation and in the baseline windows, and subtracting the real and imaginary components by vector subtraction. The merits of this method are discussed in Guinan (Guinan Jr. J. , 2006) and a far more robust MOC signal can be obtained when using a combination of phase and amplitude changes as a measure of MOC inhibition.

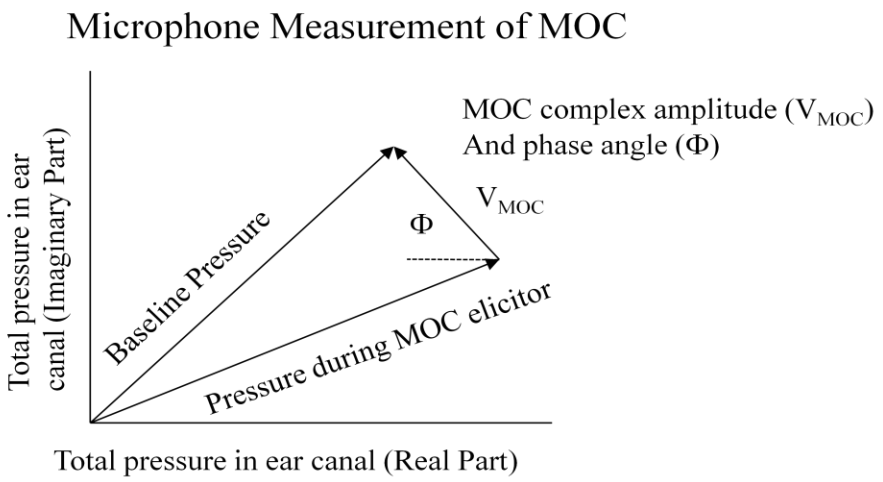

Figure 17: Method for measuring MOC inhibition of OAE using vector subtraction. Total pressure in the ipsilateral ear is first measured in a baseline window, while presenting a tone to the ear, which evokes the SF OAE. While the tone is still being presented, an MOC elicitor is presented (noise) either ipsilaterally, contralaterally, or bilaterally. If the MOC elicitor is presented contralaterally, then the effect of the MOC elicitor on the SF OAE can be measured during the presentation of the elicitor. If the MOC is presented ipsilaterally or bilaterally, then the MOC must be measured after the elicitor has turned off – within a post-elicitor window. The MOC complex amplitude (A) and phase angle (Φ) are calculated by vector-subtracting the total pressure measured in the elicitor or post-elicitor measurement window from the total pressure measured in a baseline window.

The MOC is inherently a noisy measurement and to obtain an accurate estimate of the amplitude, phase, and timing of the MOC response, it is necessary to (1) remove outliers caused by patient motion, (2) average responses over multiple presentations, and finally (3) accurately estimate the size of the MOC response. Outliers were removed by taking the FFT of the total pressure measured in the ear canal during the baseline as well as in a post-elicitor window extending from 40 ms to 120 ms after the elicitor had turned off for 50 presentations, and measuring the mean complex magnitude and the standard deviation of the response at the frequency of the probe. Outliers were defined as having complex amplitudes of more than one standard deviation outside of the mean and also having a signal-to-noise ratio of less than 3 dB. These outliers were removed from further analysis. After the outliers were removed, the remaining post-elicitor time-series waveforms were averaged. The averaged waveform was digitally heterodyned. The digital heterodyne (Kim, Dorn, Neely, & Gorga, 2001) enabled the extraction of the temporal envelope of a time-varying signal within a narrow frequency region.

First an FFT was done on the residual and 1000 spectral components about the probe frequency were selected and shifted downward to be centered at 0 Hz. The shifted signal was then filtered with a 1500 tap FIR filter with 90 Hz cut-off frequency, 34 ms of splatter, and zero phase delay. Finally a Blackman window was applied and then the output was converted back to the time domain with an inverse FFT. The amplitude of the MOC response (V_{MOC}), the latency (L_{MOC}), and the turn-on / turn-off time (τ_{MOC}) were estimated from the parameters of the curve fit to Equation 2. A summary of this signal extraction method is given in Figure 18 below.

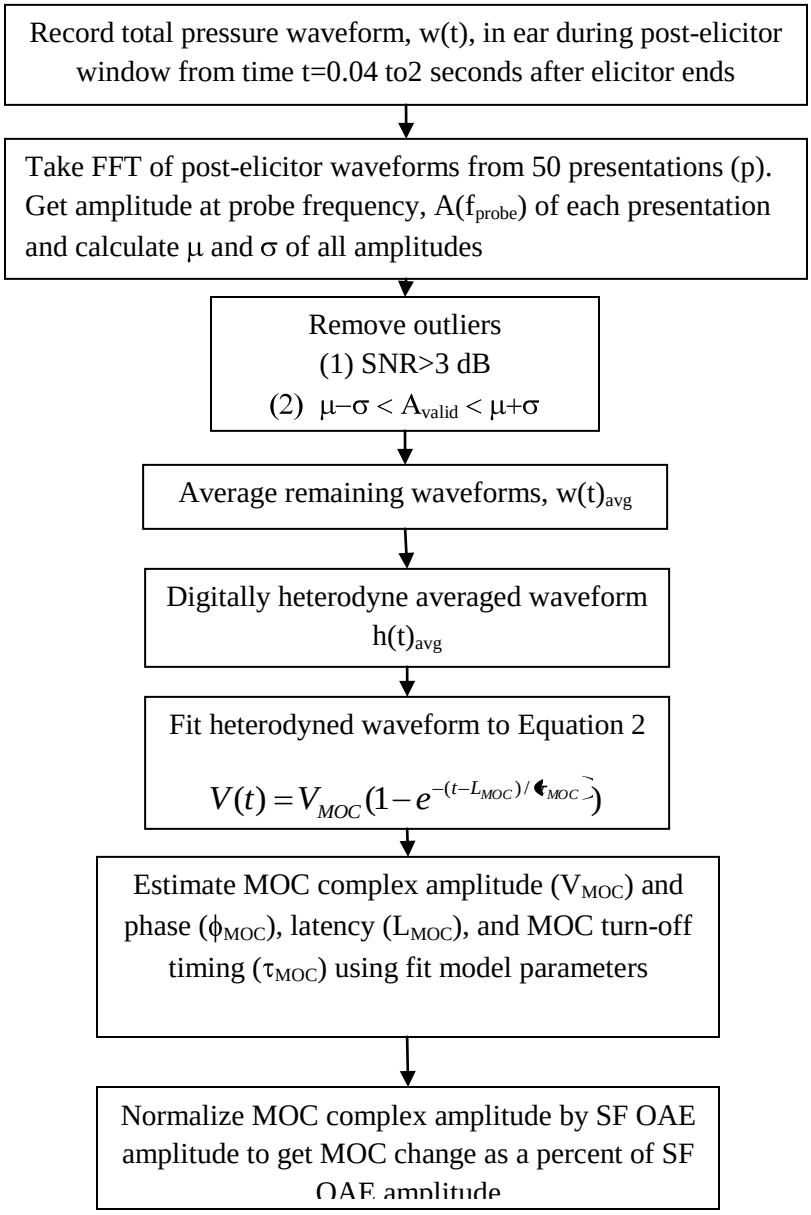

Figure 18: MOC amplitude and timing estimation methods

Plots from one of the subjects are shown in Figure 19 below. The average total pressure measured in the ipsilateral ear in response to a 55 dB pure tone is shown both during MOC elicitation and without it in (a). The digitally heterodyned waveform is shown in (b), and it is now apparent that when the MOC noise elicitor is being presented to the contralateral ear, the total pressure measured in the ipsilateral ear is being inhibited. Finally, in (c) we show the results of a curve-fit that demonstrates how amplitude, rise-time and latency are extracted from the waveform.

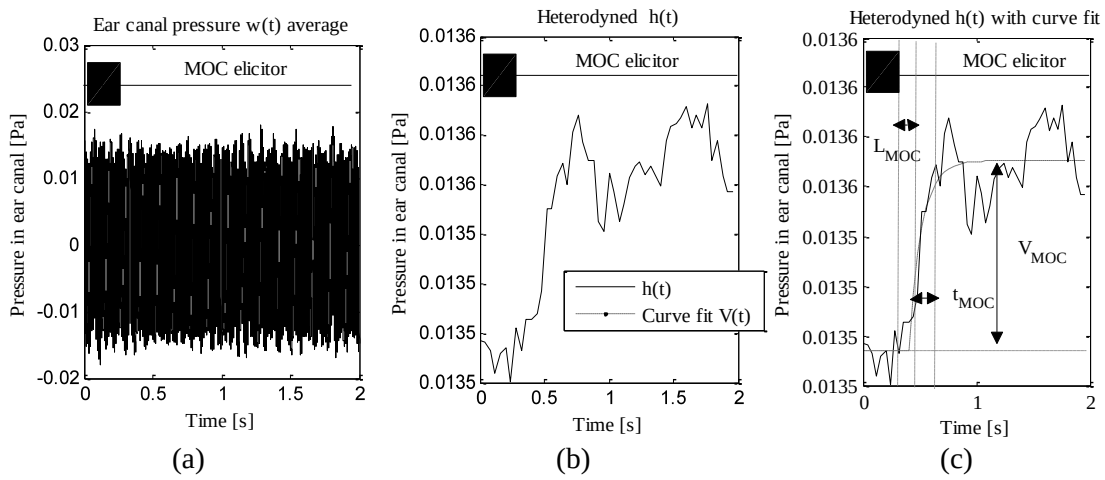

Figure 19: Signal processing of MOC waveforms. (a) Average of ear canal pressure recorded in the ipsilateral ear in response to a 55 dB SPL pure tone both during an MOC noise elicitor presentation in the contralateral ear and without this presentation. Notice that the MOC noise elicitor turns off at 0.4 seconds. The inhibition of the ear canal pressure during MOC elicitation is impossible to detect prior to the heterodyning. (b) Average digital heterodyne, the MOC inhibition of the ear canal pressure during the noise elicitor presentation can be observed. When the MOC elicitor is turned off, the ear pressure returns to its baseline level. (c) Results of fitting an exponential curve to heterodyned waveform to extract MOC amplitude, rise-time, and latency measures.

The steps involving curve-fitting the MOC waveform have not been traditionally used before and therefore it is important to compare the curve-fit method of amplitude estimation with other published methods of extracting the MOC amplitude. An alternative to the curve-fit method of extracting the MOC amplitude is described by Guinan (2006) in which the magnitude within a post-elicitor window is calculated and this magnitude is subtracted from the magnitude of the total pressure in the ear during a baseline window. The baseline window is a time during which there is no elicitor present. In the timing diagram in Figure 16, baseline windows and post-elicitor windows are given in Table 2 below.

Elicitor	Processing window [s]	Baseline window [s]
Ipsilateral	2.04-2.12	5-6
Contralateral	5-6	7-8
Bilateral	10.04-10.12	11-12

Table 2: Processing windows and baseline windows for FFT-method of MOC analysis. Notice that the contralateral processing window occurs during the presentation of the elicitor. This is because effects of the contralateral MOC elicitor on the SF OAE measured in the ipsilateral ear can be measured simultaneously. Whereas effects of the ipsilateral and bilateral MOC elicitor on the SF OAE evoked in the ipsilateral ear must be measured after the elicitor has been turned off.

The post-elicitor window begins 40 ms after the elicitor stops for both the ipsilateral and bilateral elicitors. This is because it is necessary to provide enough time for multi-tone suppression effects to subside after the elicitor is turned off for the condition where the elicitor is presented to the same ear in which the probe tone is presented. However, when the elicitor is presented to the contralateral ear, the measurement can be made during the same time that the elicitor is presented. In Figure 20 below, a comparison of curve-fit-based versus FFT-based MOC amplitude estimates are shown.

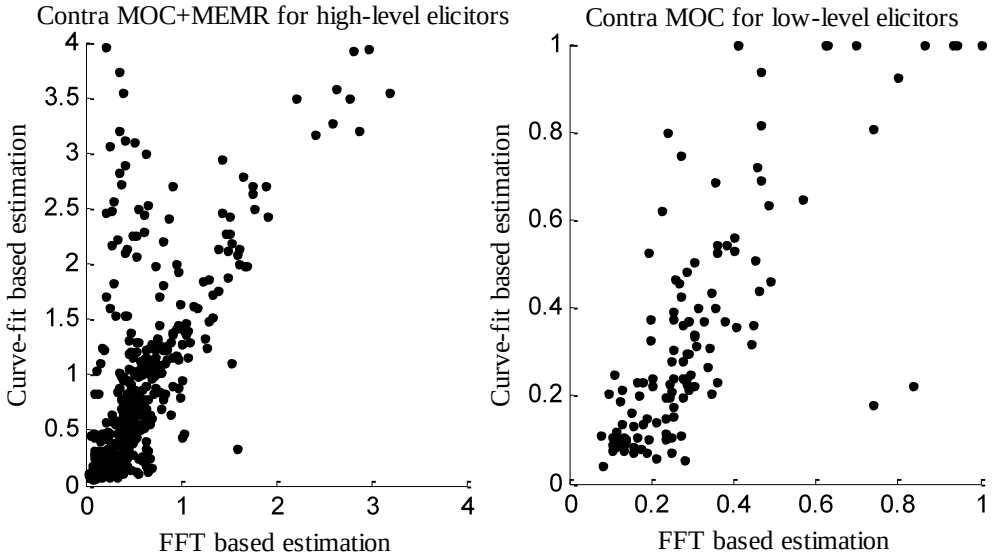

Figure 20: Comparison of curve-fit vs. FFT method of contralateral MOC amplitude estimate. Amplitudes are shown as a fraction of the size of the SF OAE amplitude. Notice that the higher-level elicitors are creating an inhibition effect that is larger than the SF OAE amplitude. This is because the MEMR creates increased input acoustical impedance to the ear canal, which is a larger change than that created by MOC activation. However, the MOC activation reduces the gain of the cochlear amplifier, and thereby only reduces the amplitude of the SF OAE.

Although there is a correlation between the curve-fit-based method and the window-based method of amplitude estimation, it is clear from the figure above that the curve-fit based amplitude estimation appears to over-estimate the amplitude relative to the window-based method of amplitude estimation. The reason why this occurs is likely due to the curve fitting to noise spikes that may occur within the post-elicitor window, causing the amplitude to be over-estimated. Because of the general lack of agreement between the curve-fitting method of amplitude estimation and the FFT window-based method of estimation, we used the more traditional FFT window-based method of amplitude estimation in all of the experiments described in the rest of this paper to estimate MOC complex amplitude. However, the rise-time and latency estimates were extracted using the curve-fit method.

2.2.7. Signal processing of SF OAE waveforms

Temporal envelopes of stimulus and SF OAE waveforms were obtained also using a digital heterodyne method for amplitude and phase extraction at the probe frequency. Within this method, the residual SF OAE waveform (P_d) is first calculated using Equation 1. An FFT is done on the residual and 1000 spectral components about the probe frequency are selected and shifted downward to be centered at 0 Hz. The shifted signal is then filtered with a 1500 tap FIR filter with 10 Hz cut-off frequency, 34 ms of splatter, and zero phase delay. Finally a Blackman window is applied and then the output is converted back to the time domain with an inverse FFT.

After the filtering and envelope extraction, the residual SF OAE waveform data was fit to the low-pass filter model equation described in section 1.11 using a non-gradient simplex optimization algorithm.

Equation 6 $$y(t) = V_{SFOAE}(1 - e^{-(t - L_{SFOAE})/\tau_{SFOAE}})$$

Fitting the residual enables the estimation of the amplitude ($V_{SF\,OAE}$), the rise time ($\tau_{SF\,OAE}$), and the latency ($L_{SF\,OAE}$). The estimated SF OAE amplitude was compared with the estimated

amplitude when measured in a coupler. Invalid data was removed from the data set according to simple exclusion criteria. The SF OAE residual was considered valid if the following two conditions were both true:

1. The estimated SF OAE amplitude in a test subject was greater than two standard deviations above the estimated SF OAE amplitude in a coupler and.
2. If the curve fit of the residual to Equation 2 had a Pierson correlation coefficient $r^2>0.5$

The signal processing is summarized in the table below.

SF OAE Signal Processing and feature extraction summary	
Step 1	Extract SF OAE residual $Pd=P_1+P_2-P_{12}$ from 20 waveforms
Step 2	Estimate SF OAE amplitude by taking FFT of each Pd. Remove outliers beyond 1 standard deviation of mean. Take mean of remaining best waveforms. Call this Pd_mean
Step 2	Convert Pd_mean to frequency domain (FFT)
Step 3	Extract 1000 spectral components about probe frequency
Step 4	Shift spectral components to 0 Hz
Step 5	Filter using 1500 tap, 90 Hz, zero phase delay, 34 ms splatter FIR filter
Step 6	Convert back to time-domain to get filtered SF OAE (Pd_filt)
Step 7	Fit Pd_filt to model $y(t)=V_{SFOAE}(1-e^{-(t-L_{SFOAE})/\tau_{SFOAE}})$ to extract amplitude ($V_{SF\ OAE}$) and latency ($L_{SF\ OAE}$) relative to the probe waveform.

Table 3: SF OAE signal processing methods.

Shown in Figure 22 are the SF OAE and probe waveforms as processed in steps 1-6.

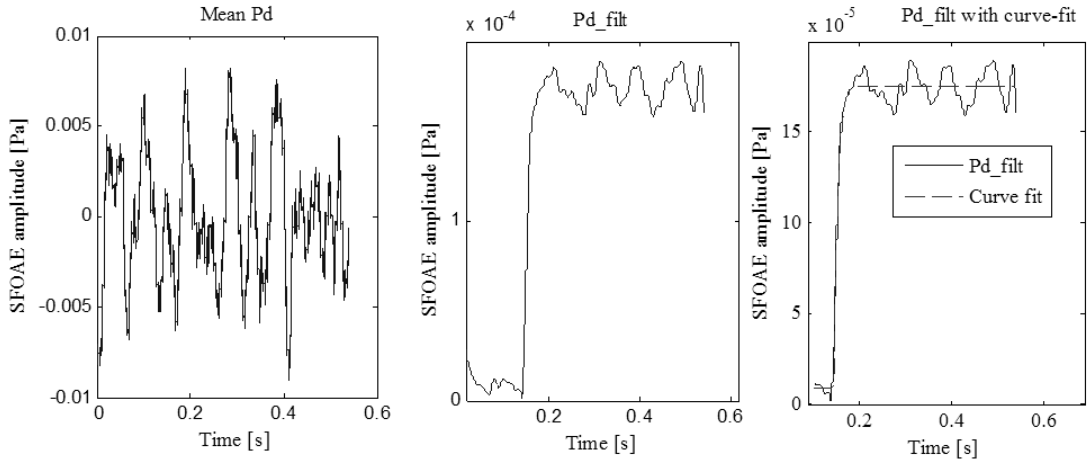

Figure 21: Plots from subject 49 showing signal processing steps of SF OAE waveforms. (a) Mean of best SF OAE (Pd) waveforms. (b) Digitally heterodyned Pd waveform (Pd_filt). (c) Pd_filt and the curve-fit, used to estimate SF OAE amplitude.

The waveform response to the probe stimuli (P2) was also processed using the steps described above. And the latency between the probe stimulus (P2) and the SF OAE waveform is shown in Figure 22 below.

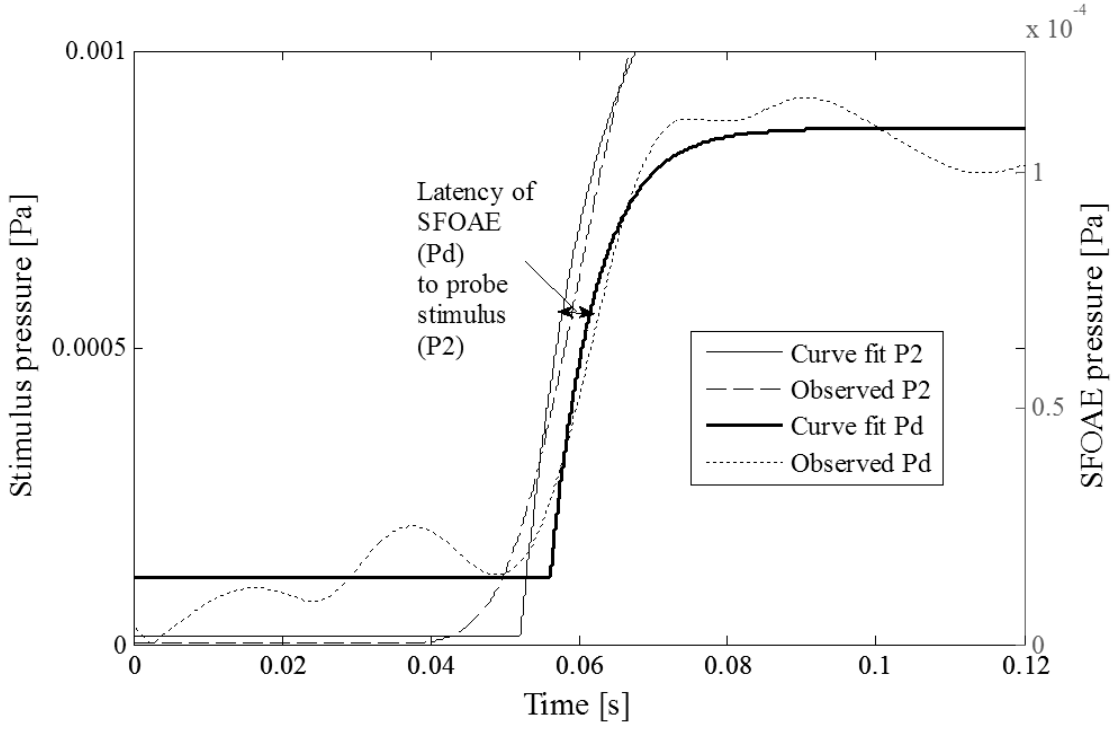

Figure 22: Example of how SF OAEs (Pd in plot above) are curve-fit to model data and latencies are calculated relative to the curve fits to the stimulus probe waveform.

The signal processing method for estimating amplitude and latency using curve-fitting as described is a new method and therefore, it is important to qualify and compare it to a more conventional method of SF OAE amplitude and latency estimation. A standard FFT-based method of estimating the SF OAE amplitude was done on SF OAE waveforms acquired and this estimation was compared with the curve-fit method of amplitude estimation. The FFT-based method of amplitude estimation simply used the magnitude of the SF OAE residual at the probe frequency as the amplitude estimate. Results showed a nearly perfect fit between the curve-fit based amplitude estimation and the FFT-based amplitude estimation as displayed in Figure 23 below.

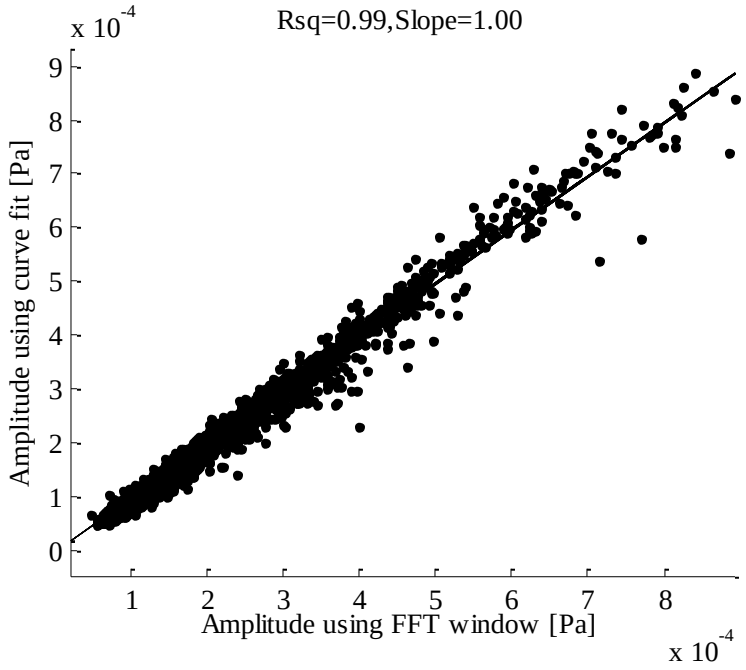

Figure 23: Results of two methods of SF OAE amplitude estimation. Results show that the curve-fit method is nearly identical to the more standard FFT window based method of amplitude estimation.

In chapter 4 we show that the latencies derived using this time-domain based method are in close agreement with prior estimates of OAE latencies using spectral methods.

2.3. Group mean analysis: normal vs. hyperglycemic conditions

An initial analysis was done to determine which of the OAE and MOC metrics correlate with blood sugar. The complex amplitude, phase, latency and rise-time of the MOC and SF OAE waveforms were calculated for subjects during normal-glycemia (blood sugar less than or equal to 160 mg/dL) and also during hyperglycemia (blood sugar greater than 160 mg/dL). For each glucose tolerance test run, a mean was calculated during normal-glycemia and hyperglycemia. A single-tail paired t-test was done to determine whether the means of each metric during normal-glycemia were statistically different than the means during hyperglycemia. Individual subject data was also analyzed and a single-tailed t-test was done to determine whether for each subject their OAE and MOC metrics were statistically different during normal glycemia vs. hyperglycemia. Statistical significance was chosen to be that in which the p-value is less than 0.05, however all p-values are reported for comparison. The metrics which were found to correlate the best with blood sugar were then used as predictors in the hyperglycemic risk analysis and continuous glucose prediction methodologies which is described in the next section.

Two groups of subjects were tested: (1) diabetic subjects and (2) nondiabetic subjects. Results from each of these experiments are shown below and then a section follows in which results from the two experiments are compared. Finally, a control experiment is discussed.

2.3.1. Diabetic subjects: correlation of OAE and MOC inhibition amplitude with glucose

All of the MOC and SF OAE data was combined and divided into two groups – hyperglycemic (blood sugar >160 mg/dL) and normal (blood sugar <= 160 mg/dL). Notice in the figure below the general trend that the MOC increases with hyperglycemia for all GTT runs and for each type of MOC elicitor tested.

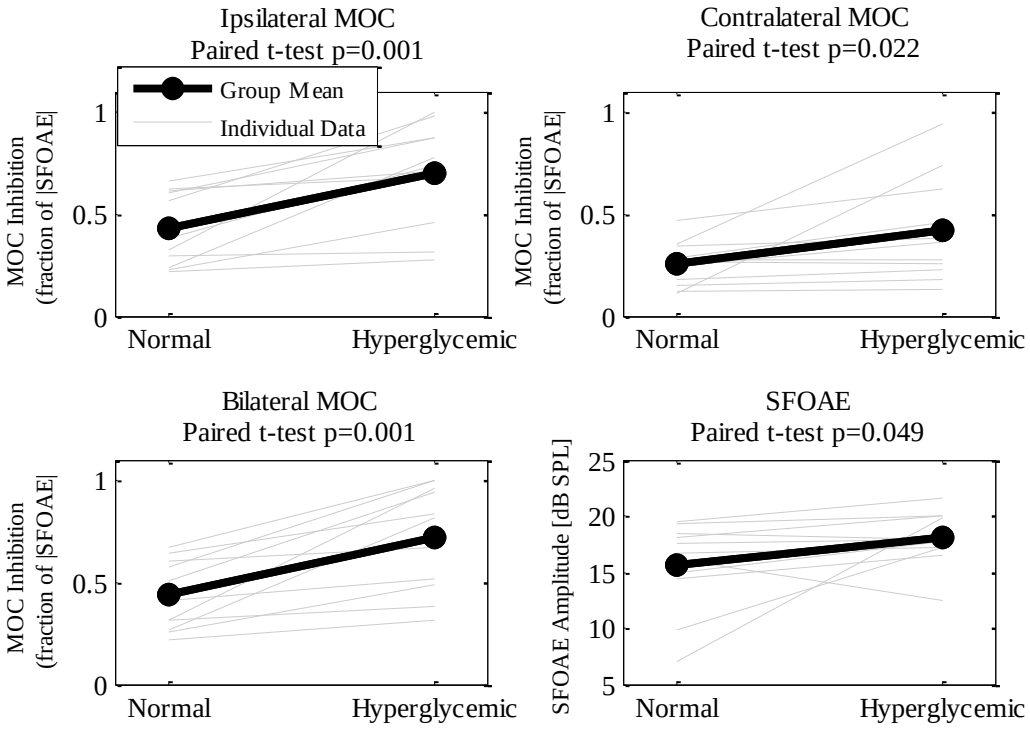

Figure 24: Mean and individual results for diabetes subjects tested using low-amplitude elicitors under normal and hyperglycemic conditions. The data shows that the mean MOC inhibition amplitude, shown as a fraction of the SF OAE amplitude, increased during hyperglycemia (blood sugar > 160 mg/dL) for all MOC elicitors; ipsilateral, contralateral and bilateral. Individual data (shown as light gray traces) demonstrates that the MOC inhibition amplitude increased during hyperglycemia for all individual subjects as well. The mean SF OAE amplitude also showed a trend of increasing during hyperglycemia, but the trend was not as consistent across all subjects.

The results show a clear trend of increasing amplitude during the hyperglycemic state for the group data. The MOC amplitude of each individual tested also showed an increase during hyperglycemia as displayed by the light gray traces in the figure above. A paired student t-test showed that the increase in MOC amplitude and SF OAE amplitude for the 11 glucose tolerance tests run was statistically significant. The significance levels are shown at the top of each plot in the figure.

Change in MOC inhibition during hyperglycemia was plotted for each individual subject's GTT run as shown in the box-plots in Figure 25, Figure 26, and Figure 27 below. For

each subject tested, the median MOC inhibition amplitude increased during hyperglycemia for each type of elicitor – ipsilateral, contralateral, and bilateral, respectively.

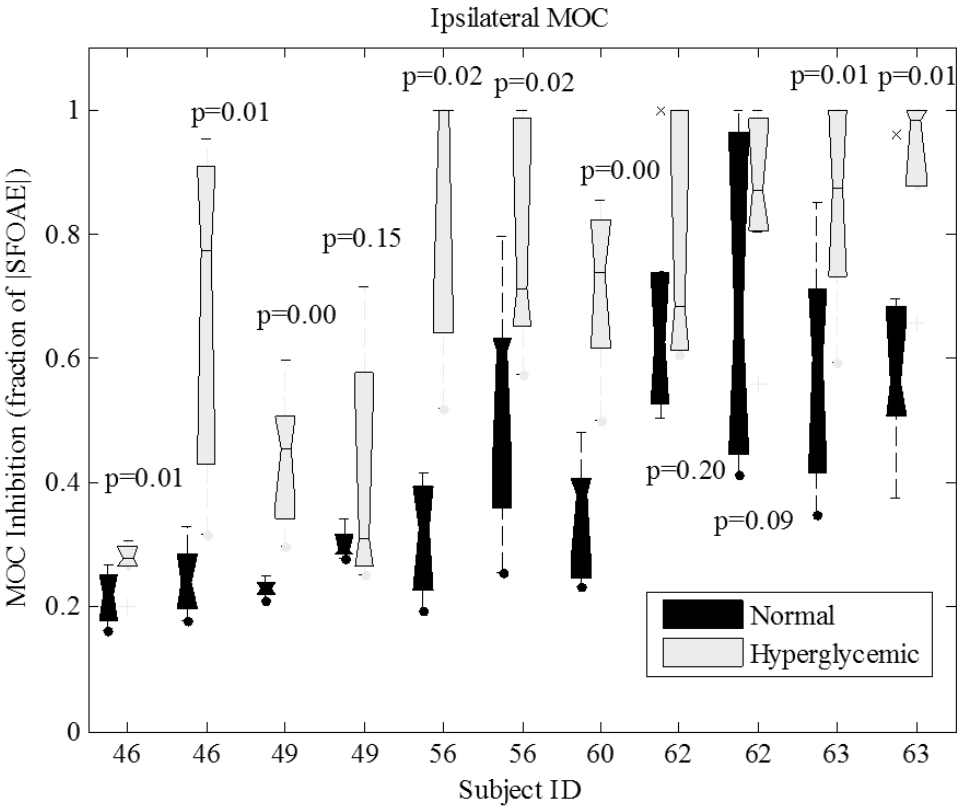

Figure 25: Ipsilateral MOC inhibition for all diabetic subjects tested. Data is divided into hyperglycemic (blood glucose >160 mg/dL) and normal. Notice that the median MOC inhibition increases for each of the subjects in the hyperglycemic condition.

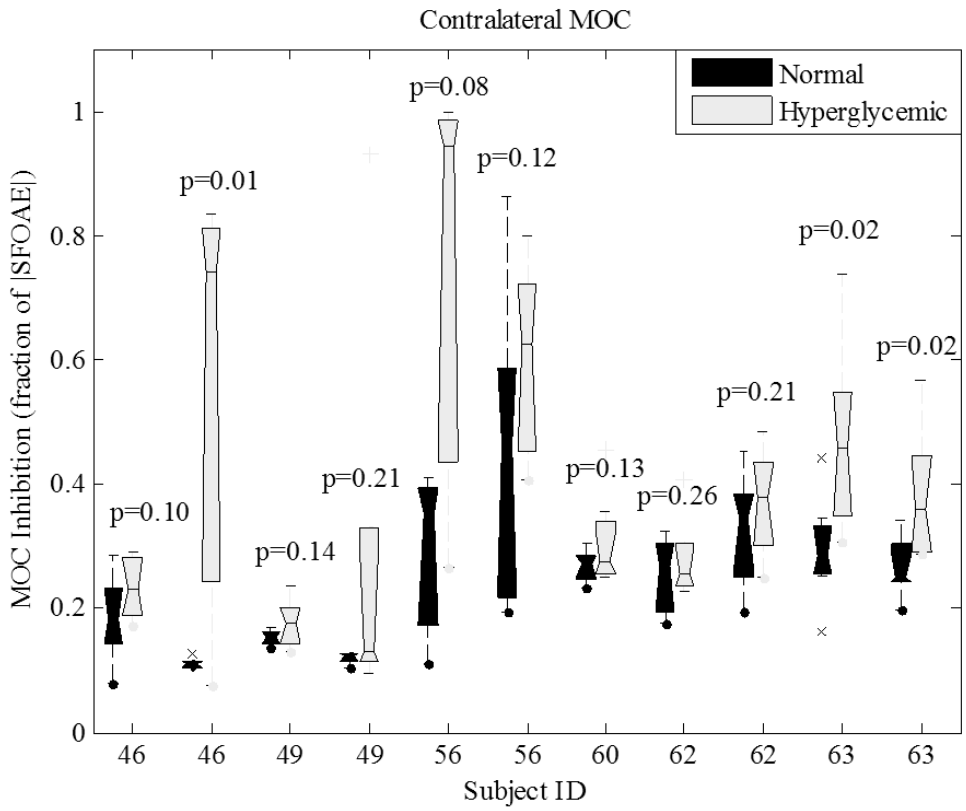

Figure 26: Contralateral MOC inhibition plotted for each subject. As with the ipsilateral MOC magnitude, the median contralateral inhibition amplitude increases for all subjects and all trials during the hyperglycemic condition.

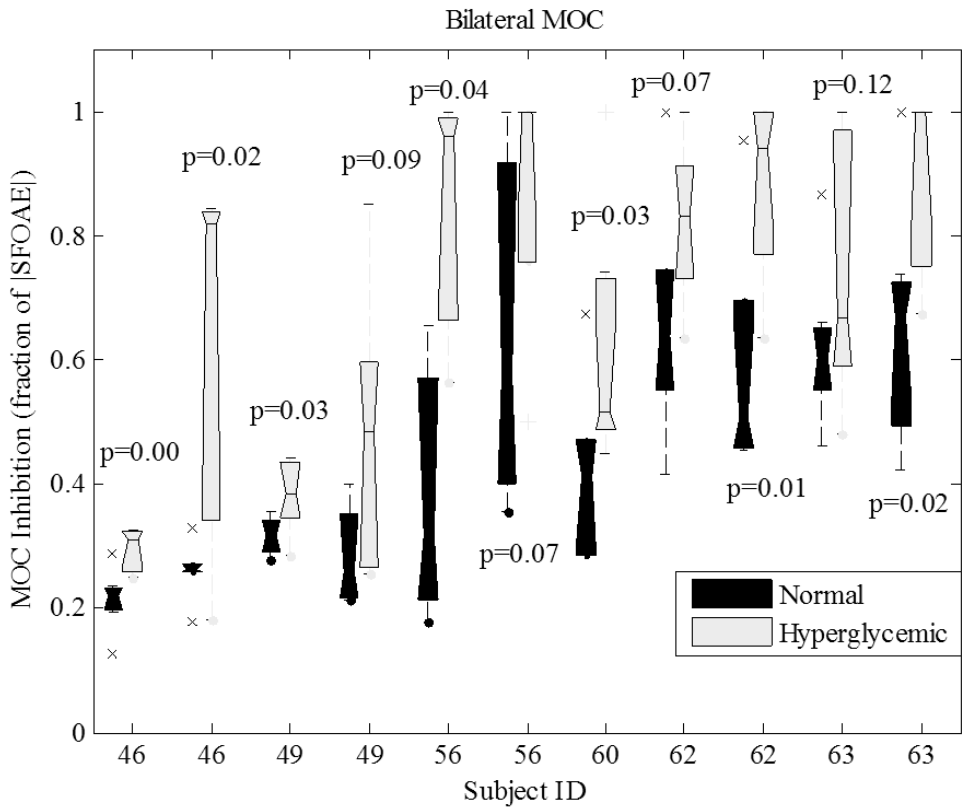

Figure 27: Bilateral MOC complex magnitude also demonstrated a positive correlation with hyperglycemia for each of the trials and across all subjects.

A student t-test was used to determine whether MOC amplitudes recorded during the hyperglycemic state (glucose > 160 mg/dL) were greater than the MOC amplitudes recorded during normal-glycemia on each individual run. The p-values for each individual t-test are shown on the box plots above. Notice that for the ipsilateral and bilateral MOC elicitors, for nearly all of the 11 GTT trials, a statistically significant p-value was determined ($p<0.05$) when testing the hypothesis that the MOC amplitude increased during hyperglycemia.

The SF OAE amplitude, while showing a group trend towards increasing during hyperglycemia (p=0.049), was not consistent across all subjects as observed below in and only displayed statistical significance in 5 out of 11 subjects.

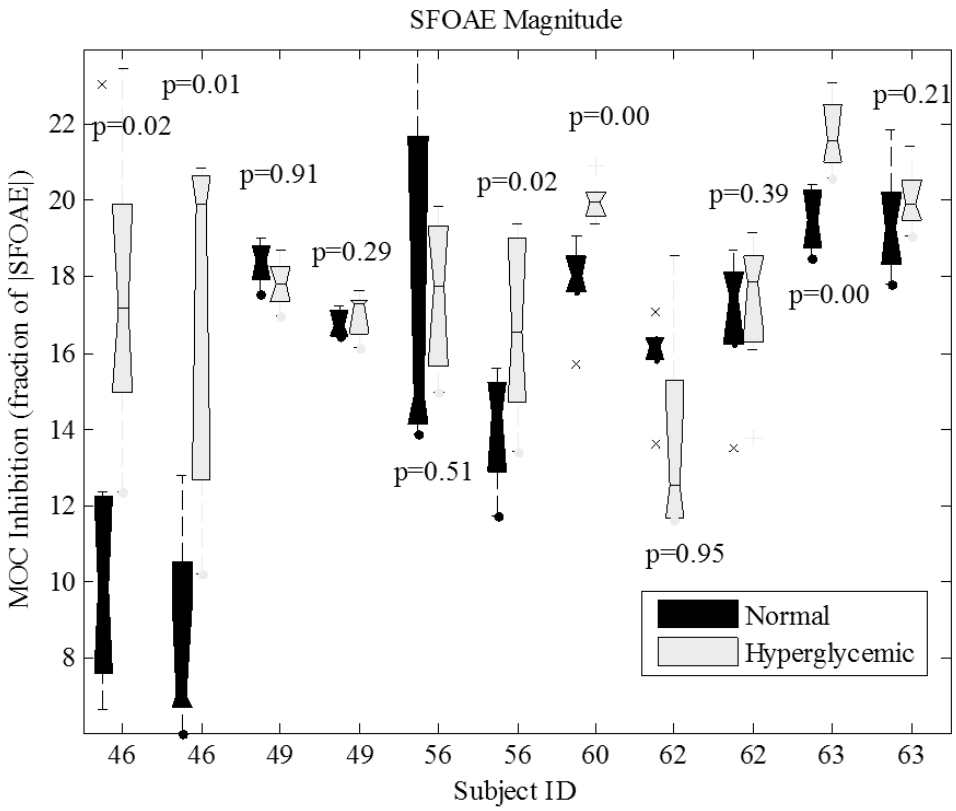

Figure 28: The SF OAE amplitude, while increasing for the majority of GTT tests during hyperglycemia, did not change as consistently or as repeatably across subjects as the MOC inhibition.

2.3.2. Glucose correlation with other MOC and SF OAE metrics

While MOC inhibition amplitude and SF OAE amplitude showed the best correlation with hyperglycemia, other metrics of the SF OAE and MOC were examined as well. Overall, the rise-time and onset latency of the SF OAE and the MOC did not show any statistically significant correlation with blood glucose. The only metric that demonstrated a weak correlation with blood glucose was the bilateral MOC complex phase angle (a description of how complex phase angle is measured is shown in Figure 17). Since the bilateral MOC phase demonstrated a weak correlation with blood glucose, it was included (along with SF OAE amplitude and MOC inhibition complex amplitude) as a potential predictor of hyperglycemia when the predictive model was constructed in later sections of this chapter.

2.3.3. Nondiabetic subjects: correlation of OAE and MOC inhibition amplitude with glucose

Non-diabetic subjects were tested using higher elicitor levels. The reason higher level elicitors were used is because this experiment was done prior to the diabetic subject experiment, and we had not yet improved our methods for measuring the MOC inhibition using lower-amplitude elicitors. The inhibition observed in the non-diabetic test subjects' ear canals caused by the presentation of noise elicitors was significantly larger and noisier, primarily due to the fact that the higher level elicitors evoke middle ear muscle reflexes, which generate larger, more variable pressure changes in the ear. The inhibition observed when presenting higher level elicitors is primarily due to changes in the input sound pressure as a result of a change in acoustical impedance when the stapedius muscle contracts as described in section 1.6.1. Overall, the change in inhibition during hyperglycemia was inconsistent as the individuals' inhibition due to noise elicitation did not consistently increase after consumption of the glucose drink as was the case for the experiment done on diabetic subjects using lower-level elicitors. However group mean MOC and SF OAE amplitudes followed a similar trend as when the lower-amplitude MOC elicitors were used on the diabetic subjects – specifically mean MOC and SF OAE amplitudes tended to increase during elevated sugar levels as shown in . It is important to note that for the nondiabetic subjects, "hyperglycemia" was defined as blood sugar exceeding 115 mg/dL. While this would never be considered clinically hyperglycemic, it allowed us to examine the affect of consuming a large amount of sugar in nondiabetic subjects, whose blood sugar oftentimes did not rise above 125 mg/dL.

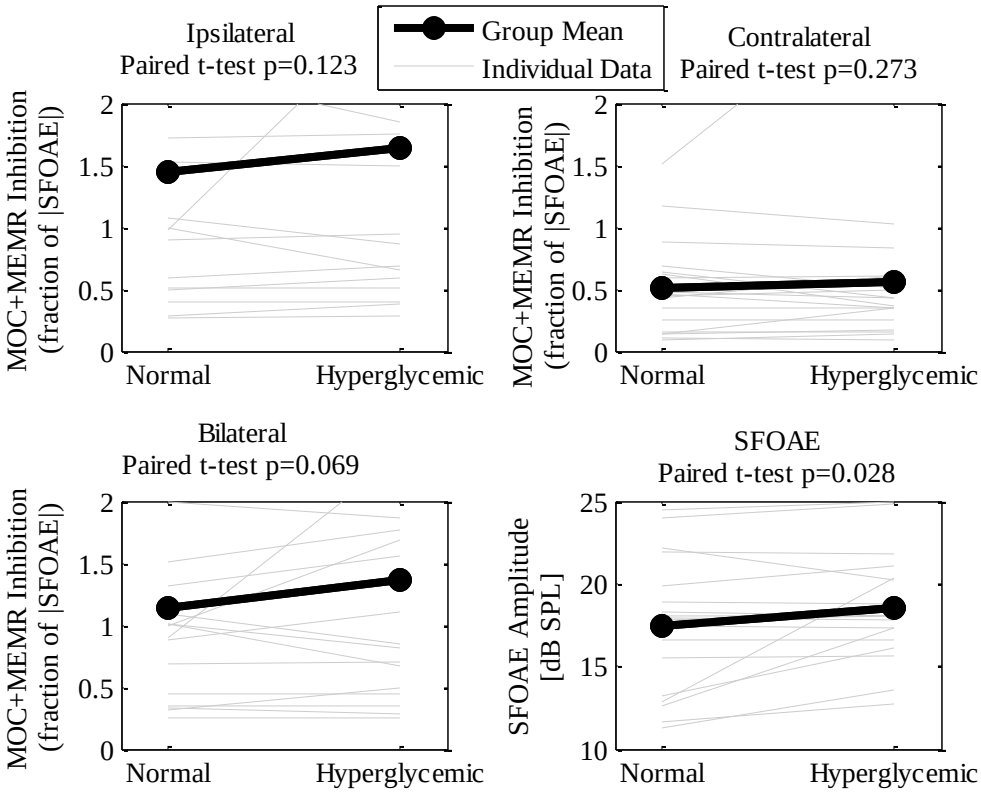

Figure 29: Group mean plots for higher-level MOC+MEMR elicitors used on non-diabetic subjects. In this test, hyperglycemia was considered > 115 mg/dL, since the subjects' blood sugar did not go up very high and setting the cut-off at 115 enabled a nearly equal number of data points in groups. Since higher-level elicitors are used, MOC and MEMR are being observed. This is why the fraction of the SF OAE is greater than 1 in some cases.

Notice in Figure 29 that the increase in MOC inhibition following consumption of the sugar is not consistent across all subjects, as it was for the nondiabetic subjects. And the mean increase in MOC inhibition amplitude is not statistically significant. However, as was observed with the diabetic subjects, the mean suppression amplitude increased on average for each elicitor and also increased for the SF OAE amplitude following consumption of the sugar.

2.3.4. Comparison between diabetic and nondiabetic subjects

When the change in suppression amplitude between hyperglycemia and normal glycemia are compared between the diabetes subjects (tested with low-amplitude MOC elicitors) and the nondiabetic subjects (tested with higher-level MOC / MEMR elicitors), we see that the change in

group mean was nearly identical (albeit slightly less for nondiabetic subjects) between the two experiments as shown in .

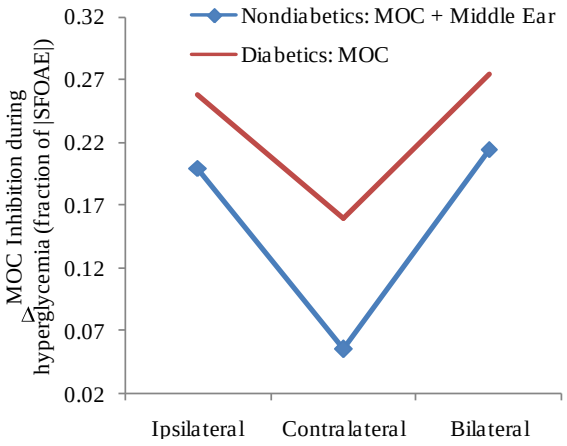

Figure 30: Change in mean MOC inhibition amplitude between hyperglycemia and normal glycemia for diabetic subjects (MOC evoked using low-level elicitors; N=11 GTTs) and non-diabetic subjects (MOC+MEMR evoked using high-level elicitors; N=18 GTTs). Notice that the change in suppression amplitude due to hyperglycemia was nearly identical for the two elicitor types and patient populations, with the diabetic subjects showing a larger increase in MOC inhibition amplitude during hyperglycemia.

While the glucose-induced increase in MOC inhibition amplitude is not statistically significant in the non-diabetic / higher elicitor experiment, several key points are important to note. First, the definition for hyperglycemia for non-diabetic subjects was 115 mg/dL instead of 160 mg/dL as was used on the diabetic subjects. Therefore, the experiment on non-diabetic subjects was measuring hyperglycemia relative to their normal fasting sugar level, but sugar levels over 115 mg/dL would never be considered hyperglycemic within a medical setting. And it may be that the mechanism that leads to changes in the MOC and SF OAE only occurs at sugar levels greater than 160mg/dL. The reason we chose 115 mg/dL as the "hyperglycemic" threshold was because in most cases non-diabetic subjects' blood glucose did not exceed 160 mg/dL after consumption of the sugar. The value of 115 mg/dL was selected because it enabled an approximate equal division of the data into a pre-glucose consumption group and post-glucose consumption group. Second, higher level elicitors were used to evoke the MOC so the MOC measurements include middle ear combined with MOC effects. The activation of the middle ear

muscle generates a response that is both larger and more variable than the MOC-alone response elicited using lower-amplitude elicitors. This increased variability due to the contraction of the middle ear muscle likely masked smaller more subtle effects of the MOC. Even so, it is interesting that the group mean of the suppression was nearly identical for the two types of elicitors, suggesting that the amplitude of the MOC response is what changes with hyperglycemia and not the middle ear muscle reflex.

The SF OAE amplitude for the non-diabetic subjects increased for the non-diabetic subjects as well as for the diabetic subjects during elevated sugar levels and the increase was statistically significant for both patient populations. The comparison between the diabetic and non-diabetic subjects change in SF OAE due to hyperglycemia is shown in Figure 31.

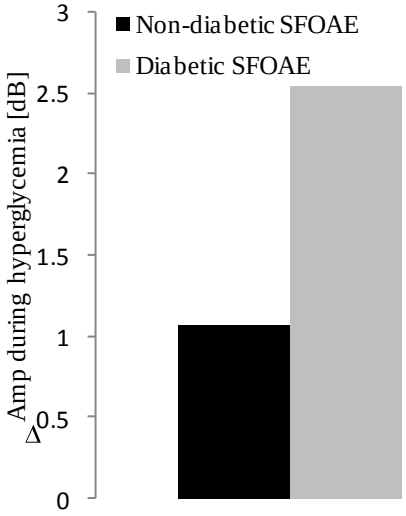

Figure 31: Change in mean SF OAE amplitude after consumption of 80 g of sugar for diabetic (N=11 GTTs) and non-diabetic (N=18 GTTs) subjects. As would be expected, changes in sugar levels were different for the non-diabetic and diabetic subjects. For non-diabetic subjects, consumption resulted in levels exceeding 115 mg/dL for all subjects, while for diabetic subjects, consumption of the sugar resulted in levels over 160 mg/dL for all subjects.

2.3.5. Diabetes control experiment: OAE and MOC inhibition change during normal-glycemia control

A control experiment was done in which three diabetic subjects followed the exact same glucose tolerance testing procedures described earlier using lower-level MOC noise elicitors with the only exception being that rather than consuming sugar 1 hour into the test, the subjects consumed 8 ounces of water. The hypothesis for this experiment was that there would be no statistical difference between the MOC measurements acquired during the control experiment and the MOC measurements acquired during the glucose tolerance test prior to the subjects' consumption of the sugar. Likewise, we hypothesized that there would be a statistically significant difference between the MOC measurements acquired during hyperglycemia and those measurements acquired during the control experiment and prior to the consumption of sugar in the GTT. Results from the control experiment for these three subjects are shown in Figure 32, Figure 33, and Figure 34.

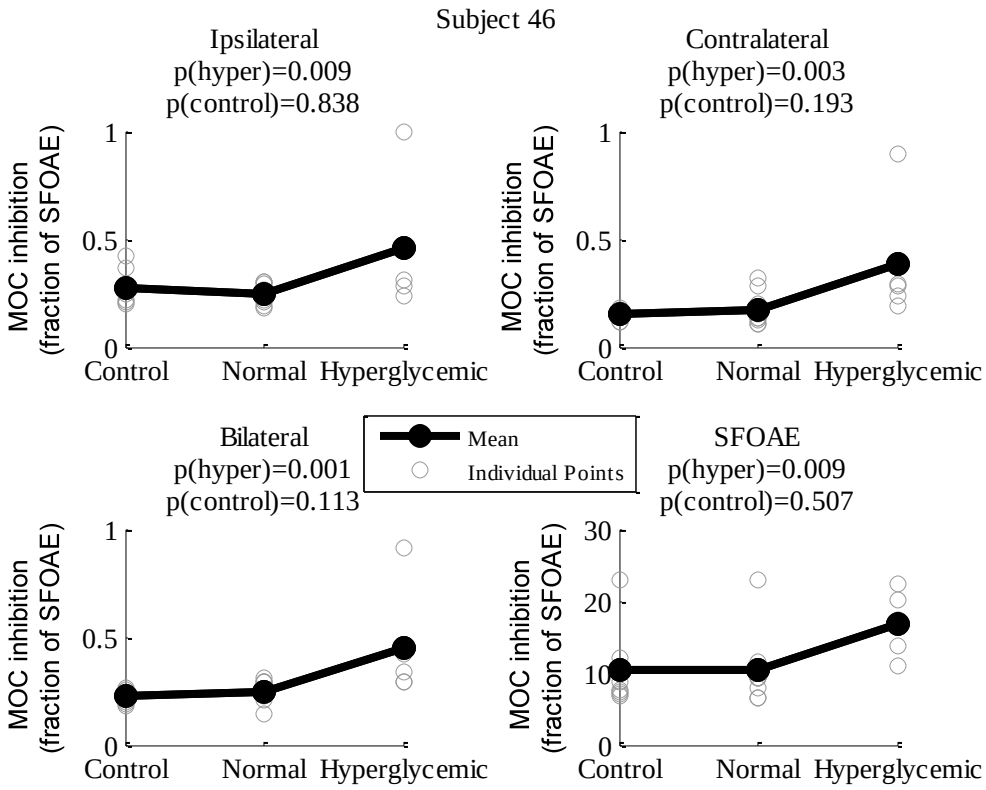

Figure 32: Subject 46 MOC and SF OAE results from control experiment ("Control"), prior to consumption of sugar during the GTT ("Normal") and after the subject's blood sugar consumed the sugar and their blood sugar exceeded 160 mg/dL ("Hyperglycemic"). For all 3 MOC elicitors, the mean MOC amplitude was larger in the hyperglycemic state than the controls.

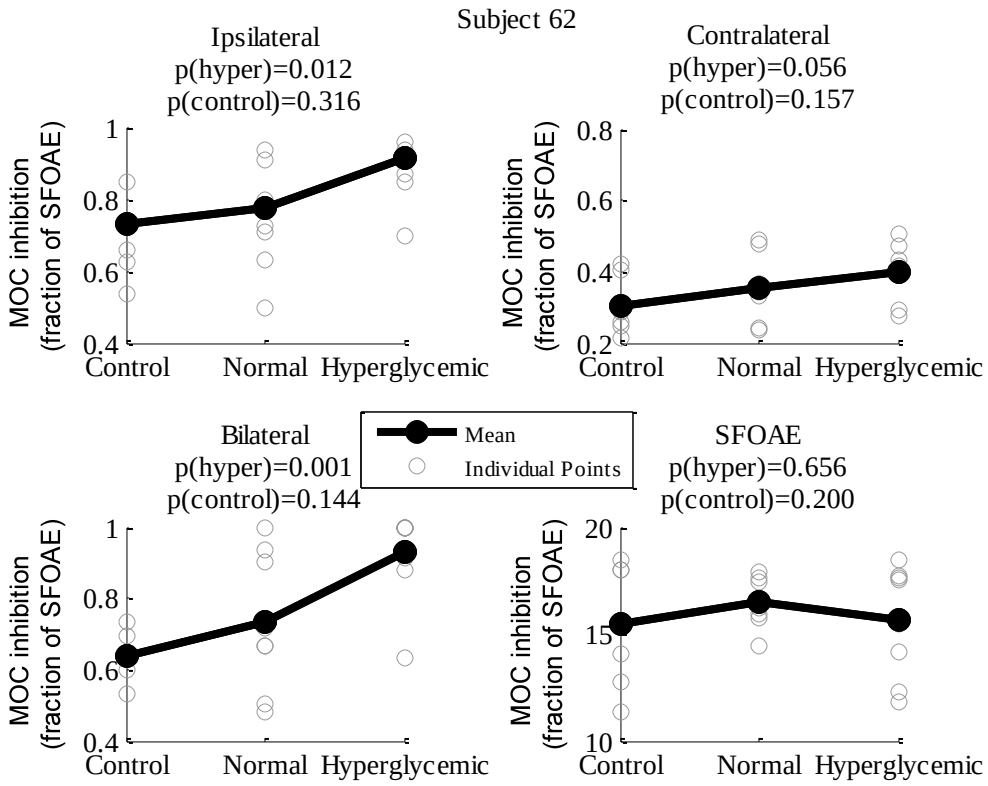

Figure 33: Subject 62 MOC and SF OAE results from control experiment and GTT. For all 3 MOC elicitors, the mean MOC amplitude was larger in the hyperglycemic state than the controls. SF OAE was not different.

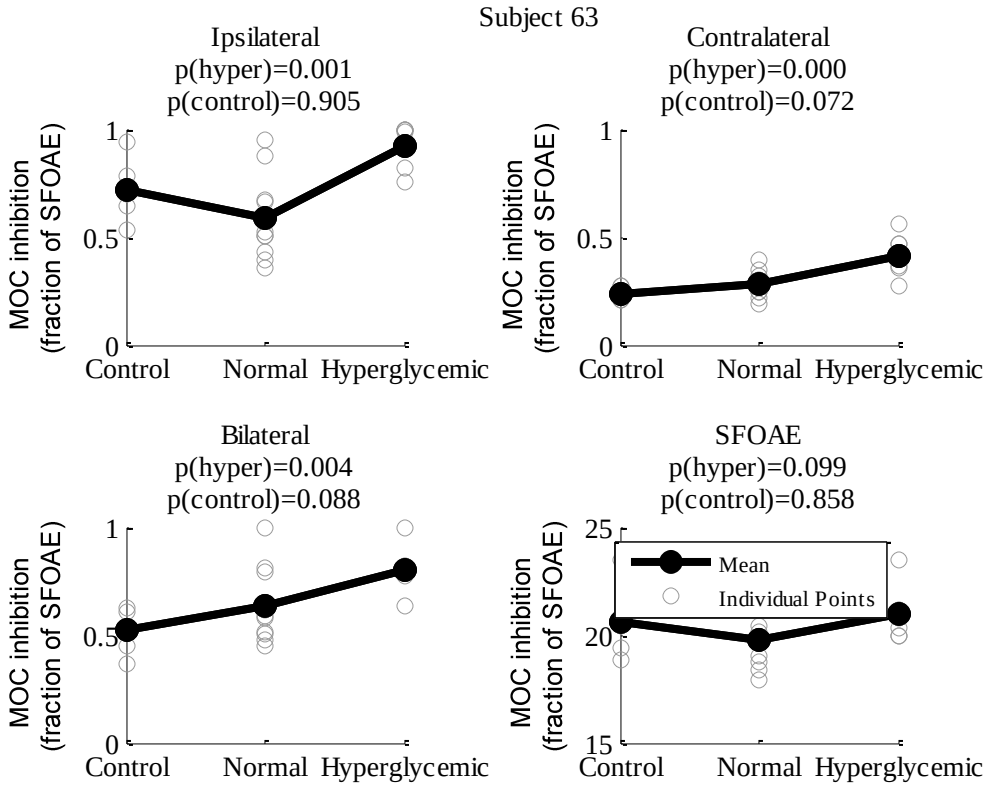

Figure 34: Subject 63 MOC and SF OAE results from control experiment and GTT. For all 3 MOC elicitors, the mean MOC amplitude was larger in the hyperglycemic state than the controls. SF OAE was not different.

Results from this test show that while the data can be somewhat variable, there is a statistically significant increase in MOC amplitude during hyperglycemia compared with both the control test run and the normal-glycemia GTT test run. The p-values shown in the figures above are from doing a single-tail t-test that tests the null hypothesis that the SF OAE amplitude and MOC inhibition amplitude during hyperglycemia is the same as during normal-glycemia and during the control experiment.

2.4. Hyperglycemic Risk Analysis (HRA) using leave-one-out cross-validated ROC analysis

A new model for predicting hyperglycemic risk based on OAE and MOC metrics was developed called Hyperglycemic Risk Analysis (HRA). HRA, derived in part from work done by

Dille et al. (2010) in which ototoxicity is estimated from OAE metrics, is based on leave-one-out receiver operating characteristic (ROC) analysis (Zweig & Campbell, 1993). This analysis was done to determine which SF OAE and MOC amplitude, timing and phase metrics were able to best predict hyperglycemic events. A hyperglycemic event for diabetic subjects was selected to be any blood sugar value over 160 mg/dL while normal-glycemia was defined as blood sugar values less than or equal to 160 mg/dL. A leave-one-out cross validation procedure was used to train and subsequently test a logistic regression model on its ability to accurately predict hyperglycemia. Predictor candidates were amplitude and timing metrics from the MOC and SF OAE waveforms, and the best ones were selected based on the Group Mean Analysis which revealed statistical correlation of the predictor with blood sugar. Each of the predictors was evaluated to determine whether it correlated with blood sugar using student t-tests as described in section 2.3. Each of the predictors was considered individually and in combination with the other metrics. An optimal combination of predictors was determined based on the area-under-the-curve criteria (AUC) whereby the AUC is defined as the average of the fraction of true positive (accurate identification of hyperglycemia) over the entire range of false positive fractions (Pepe, 2003). The combination of predictors that achieved the highest AUC was selected initially; this set of predictors was termed the P_{max}. Error bars for the AUCs for each combination of predictors were calculated using a boot-strap method in which the classical approach of sample-and-replace was used to estimate the error (Effron & Tibshirani, 1994). The combination that included the fewest number of predictors to yield an AUC that fell within one standard error of the AUC calculated using P_{max} was selected as the optimal set of predictors; this set of predictors was termed $P_{opt.}$ This model selection criteria is called the "one standard error rule" (Hastie, Tishirani, & Friedman, 2009) and is used to find the simplest model with the fewest predictors for classifying an event. Once the optimal set of predictors was determined, the final model was trained on the entire data set and this model is what is provided as the optimal predictor of hyperglycemia. The entire method is summarized below in Figure 35.

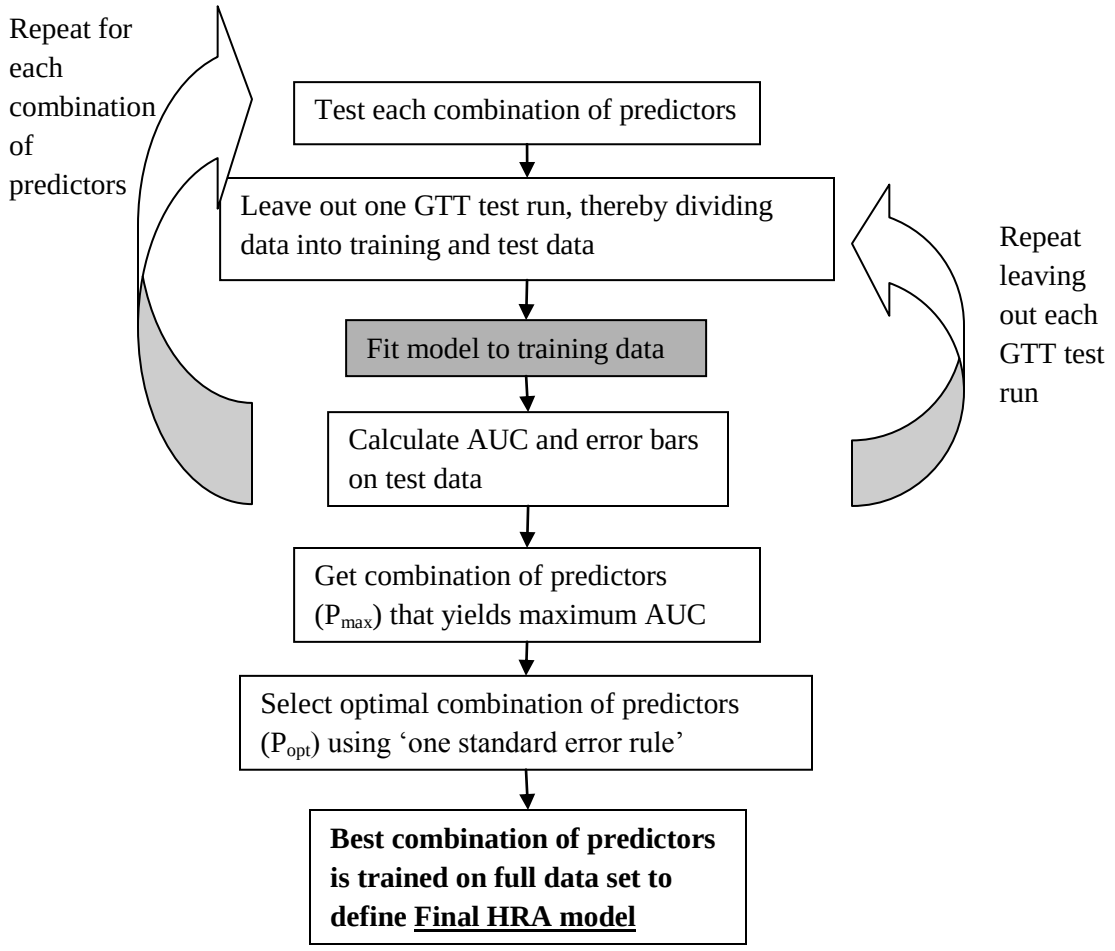

Figure 35: Leave-one-out ROC model identification methods. This method is derived from a similar model selection (Dille, McMillan, Reavis, Jacobs, Fausti, & Konrad-Martin, 2010) in which ototoxicity damage was being estimated using high frequency OAE metrics. The shaded box indicates a step that is further extended using mixed effects as described in the next section.

2.4.1. Hyperglycemia Risk Analysis (HRA) prediction on diabetes group data

A statistical analysis was done to determine whether the ipsilateral, contralateral, and MOC inhibition complex amplitudes, SF OAE amplitude and bilateral MOC phase angle could be used to accurately predict hyperglycemia in the diabetes subjects' group data. These were the five metrics that were found to statistically correlate with hyperglycemia for diabetic subjects as shown earlier this chapter. The hyperglycemic risk analysis (HRA), as described under section 2.4 demonstrates a reasonably good method for estimating hyperglycemia. The optimal

predictors were selected using the model selection technique described in section 2.4 and the one-standard deviation rule (Hastie, Tishirani, & Friedman, 2009). A plot of AUC vs. the number of predictors used to estimate hyperglycemia is given below.

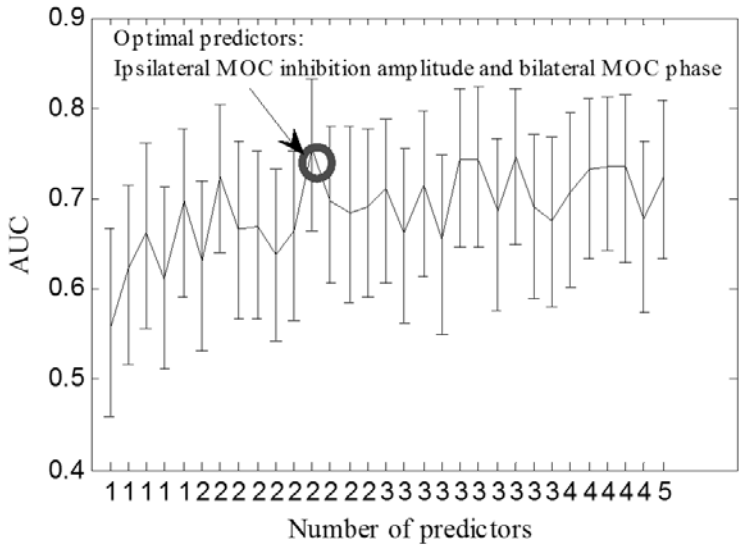

Figure 36: Model finding results for estimating the optimal predictors for estimating hyperglycemia. In the analysis shown above, five predictors were evaluated; (1) ipsilateral MOC inhibition complex amplitude, (2) contralateral MOC inhibition complex amplitude, (3) bilateral inhibition complex amplitude, (4) SF OAE amplitude, (5) bilateral MOC complex phase angle. All combinations of these 5 predictors were evaluated. The optimal predictor set (in this case ipsilateral MOC magnitude and bilateral MOC phase) was found based on largest AUC with a minimal number of predictors as defined by the 1-standard deviation rule (Hastie, Tishirani, & Friedman, 2009).

The ROC curve with error bars for the optimal set of predictors (P_{opt}) determined using the methods described in section 2.4 is shown below in Figure 37.

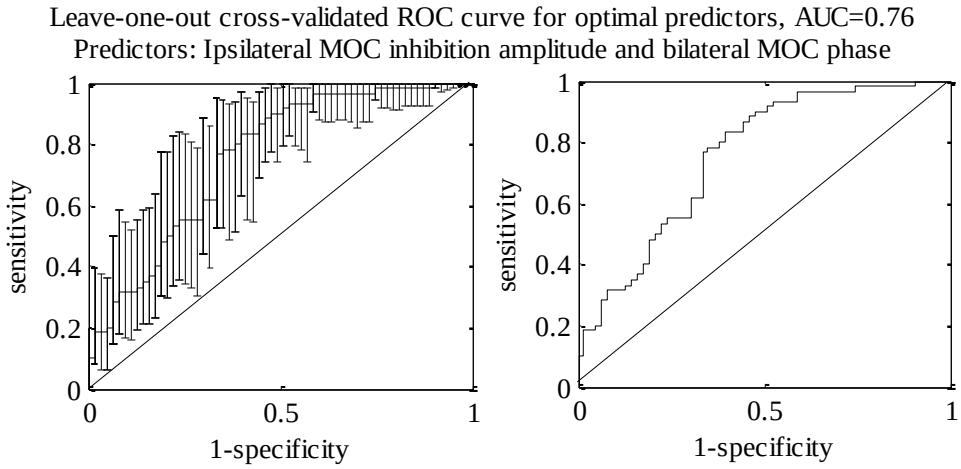

Figure 37: ROC curve for optimal predictors of hyperglycemia (blood glucose > 160 mg/dL) obtained using leave-one-out cross-validation both with and without error bars. The best predictor combination was found to be ipsilateral MOC inhibition complex amplitude and bilateral MOC complex phase angle. Error bars were determined using bootstrap sample-with-replacement. Leave-one-out AUC is 0.76.

Results from the HRA reveal that the ipsilateral MOC inhibition complex amplitude and bilateral phase angle are the optimal predictors of hyperglycemia.

2.4.2. HRA extended to include mixed effects

The ROC-based HRA method described in the previous section was extended and improved to include random and fixed effects so that inter-subject variability could be accounted for and ultimately integrated out of the model.

Model parameters were fit while evaluating random (subject-specific) and fixed (group) effects of glucose on the MOC and SF OAE metrics. The mixed effects model is shown in Equation 7.

Equation 7 $$G_p(\vec{P}) = G_f + \vec{G}_r \vec{s} + \vec{P}\vec{M}$$

In this model, $G_p(\ )$ is the model estimate of glucose for a given vector of predictors and test subject ( ) including both fixed (group) and random (subject-specific) effects. is the fixed effect glucose estimate when subject and predictor effects are zero. Likewise, is vector of

random effects for each subject when all other predictor effects are zero. is the vector of model parameters that are found during the optimization process and they represent the amount that any predictor contributes to the prediction of hyperglycemia. The predicted glucose $G_p(\ )$ is solved for using maximum-likelihood and a simplex-based optimization algorithm. The statistical significance of each model parameter solved for is determined by calculating the p-value.

In Figure 35, the step labeled as a shaded box and titled "Fit model to training data" was changed from a simple general linear model fit to a fixed effects model fit. Within the fixed effects model fit, subject-specific random effects were estimated during the training and these were included in the prediction model during model evaluation. We ensured that there was both training and evaluation data available for estimating the random effects by designing the experiment such that all subjects tested were given two glucose tolerance tests on two separate days. Therefore, in the total data set, there were two GTTs for each subject. Therefore, when the leave-one-out analysis was performed, one GTT test run was removed from the data set, but there remained another GTT for the removed subject on which the random effects for that subject could be estimated. For the diabetes test set, one of the subjects was unable to return for the second GTT and so this subject's random effects were not estimated and the fixed effect model was used instead to evaluate the model when this subject's GTT data was the one left out.

2.4.3. Mixed effects analysis results

The AUC improved for nearly all predictor sets when using the HRA extended with mixed effects over HRA alone. Figure 38 below shows how the AUC improved between the mixed effects model prediction vs. standard model prediction considering various combinations of the 5 best predictors.

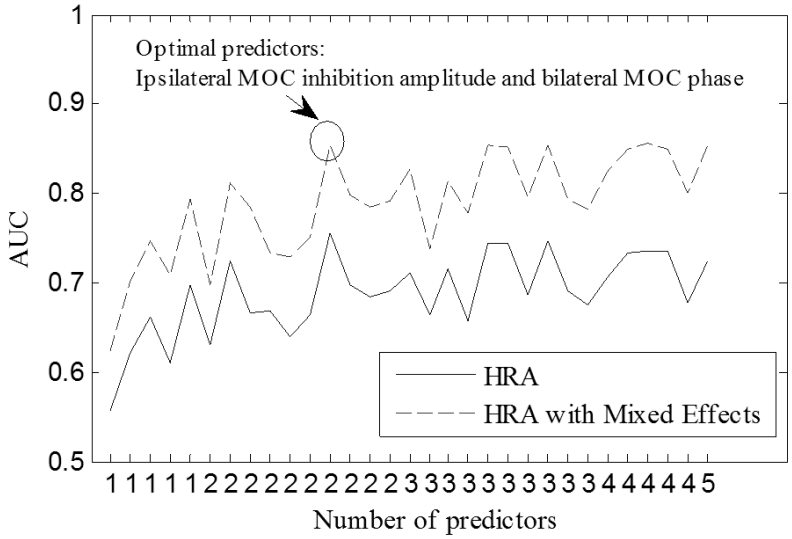

Figure 38: Improvement in AUC for mixed effects model extension of HRA. The optimal predictors of hyperglycemia are still ipsilateral MOC inhibition amplitude and bilateral MOC phase. However, the area under the curve has improved from 0.76 to 0.85 for this optimal predictor set.

It was found as in the previous HRA analysis that ipsilateral MOC inhibition amplitude and bilateral MOC phase are the optimal predictor set when considering both maximum AUC and smallest number of predictors as the selection criteria. The predictor value plotted in Figure 40 is a linear combination of the optimal predictors as shown in Equation 8 below.

Equation 8

$$P_{ME}(V_{ipsi\_amp}, V_{bil\_phase}, s) = M_f + \overrightarrow{M}_r \vec{s} + M_{ipsi\_amp} V_{ipsi\_amp} + M_{bil\_phas} V_{bil\_phase}$$

In this equation, P_{ME} is the outcome predictor value using mixed effects, V_{ipsi_amp} is the ipsilateral MOC inhibition amplitude, V_{bil_phase} is the bilateral MOC phase value, s is the subject, M_f is the fixed effect intercept value, M_r is the random effect intercept vector representing subject variability, and M_{ipsi_amp} and M_{bil_phase} are the model parameters representing the contribution of ipsilateral MOC amplitude and bilateral MOC phase to the outcome predictor value. By setting the predictor value to about PME=0.4, estimation of hyperglycemia will be 83% with a false positive rate of 23% as in the histogram plot of Figure 40.

The equation for calculating the predictor value without mixed effects is given in Equation 9below. It is identical to Equation 8 with the only exception being that it doesn't include the random effects term.

Equation 9

$$P(V_{ipsi\_amp}, V_{bil\_phase}) = M_f + M_{ipsi\_amp}V_{ipsi\_amp} + M_{bil\_phas}V_{bil\_phase}$$

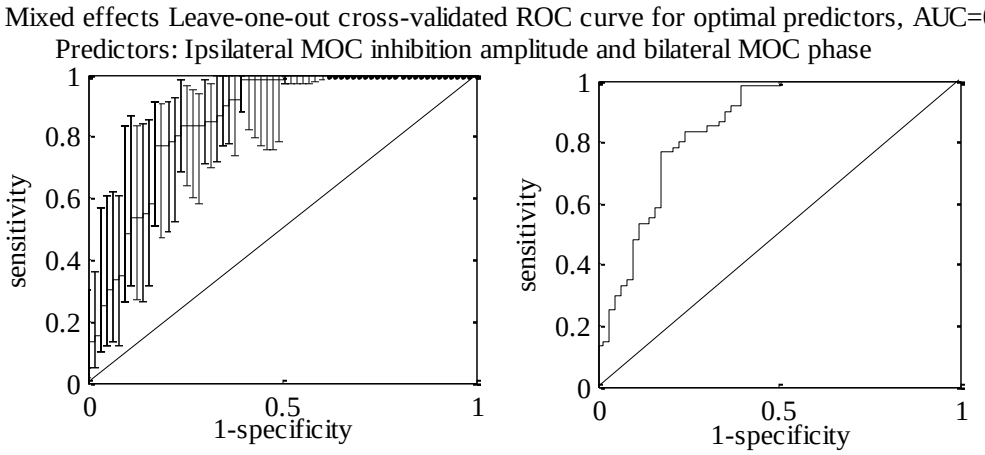

Figure 39: HRA extended using mixed effects model. The optimal predictors were again found to be ipsilateral MOC inhibition amplitude and bilateral MOC phase and the final leave-one-out area under the curve was found to be 0.85, which was a significant improvement over the model determined without using mixed effects.

The histograms below show how the predictor mean and standard deviation varies under both mixed effects (a) and non mixed effects (b) conditions.

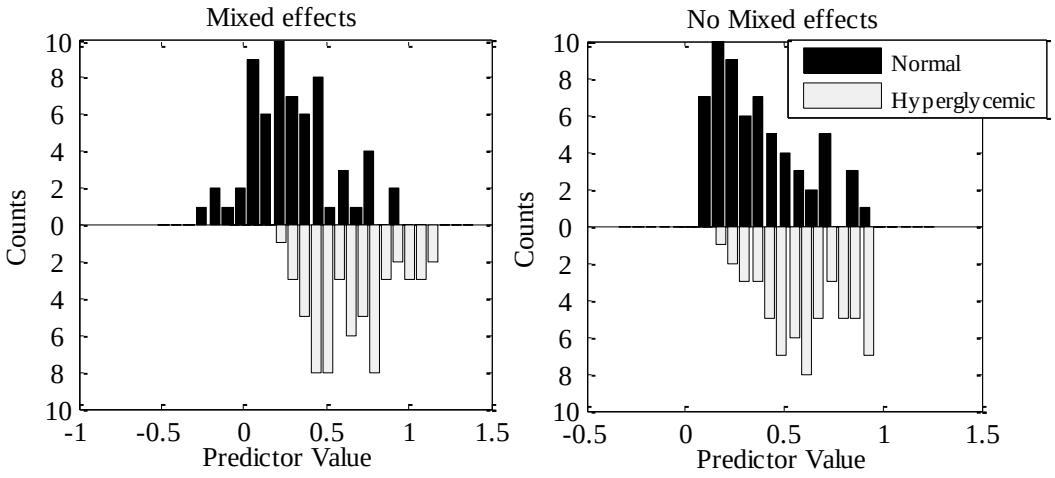

Figure 40: Model predictor results using both mixed effects and no mixed effects. The predictor value is a linear combination of ipsilateral MOC inhibition amplitude and bilateral MOC phase. The non mixed effects model result shown was fit using the entire data set, while the mixed effects model predictor is shown for the model trained on the leave-one-out cross validated model. There was a significant improvement in separating the hyperglycemic from the normal when using mixed effects.

Parameter Name	HRA Model Parameter value	p-value	Mixed Effects HRA Model Parameter value	p-value
Fixed effect intercept (M_f)	-2.75	<0.001	-0.2882	0.03
Ipsilateral MOC Inhibition amplitude (M_{psi_amp})	4.5	<0.001	1.35	<0.001
Bilateral MOC Inhibition phase (M_{bil_phase})	1.47	0.0018	0.28	<0.001

Table 4: Model parameters from HRA and HRA extended with mixed effects. Model predicts hyperglycemia based on optimal predictors found to be ipsilateral MOC inhibition amplitude and bilateral MOC phase.

2.5. Continuous glucose prediction using mixed effects

In addition to predicting hyperglycemia, as was done in HRA, continuous blood sugar was also estimated using the two optimal predictors found in the HRA procedures; ipsilateral MOC inhibition amplitude and bilateral MOC phase angle. The procedure for estimating continuous blood sugar levels is similar to what was described in HRA, with the exception that continuous regression rather than logistic regression is used to estimate the model parameters.

Shown below in the top of Figure 41 is a display of how the test subjects' blood glucose changed during each of the GTTs (light gray) as well as the mean across all subjects. The bottom trace of Figure 41 shows the continuous model prediction using the optimal predictors. Results shown are based on prediction done using leave-one-out cross validation, such that prediction is never done using data that was also used in training. This is identical to how prediction was shown earlier within the HRA results.

The equation used to predict continuous blood glucose using the optimal predictors is given in Equation 10.

Equation 10

$$G(V_{ipsi\_amp}, V_{bil\_phase}, s) = M_f + \overrightarrow{M}_r \vec{s} + M_{ipsi\_amp} V_{ipsi\_amp} + M_{bil\_phas} V_{bil\_phase}$$

The definitions for the model parameters are the same as those defined for Equation 8. In this case G is the blood glucose value estimated by the model. Model parameters from this continuous prediction analysis are given in Table 5 below.

Parameter Name	Parameter Value	p-value
Fixed effect intercept (M_f)	57.1 mg/dL	0.035
Ipsilateral MOC Inhibition amplitude (M_{psi_amp})	231.53 mg/dL/(Pa/Pa)	<0.001
Bilateral MOC Inhibition phase (M_{bil_phase})	32.24 mg/dL / rad	0.021

Table 5: Continuous prediction of blood sugar using optimal predictors (ipsilateral MOC inhibition amplitude and bilateral MOC phase angle).

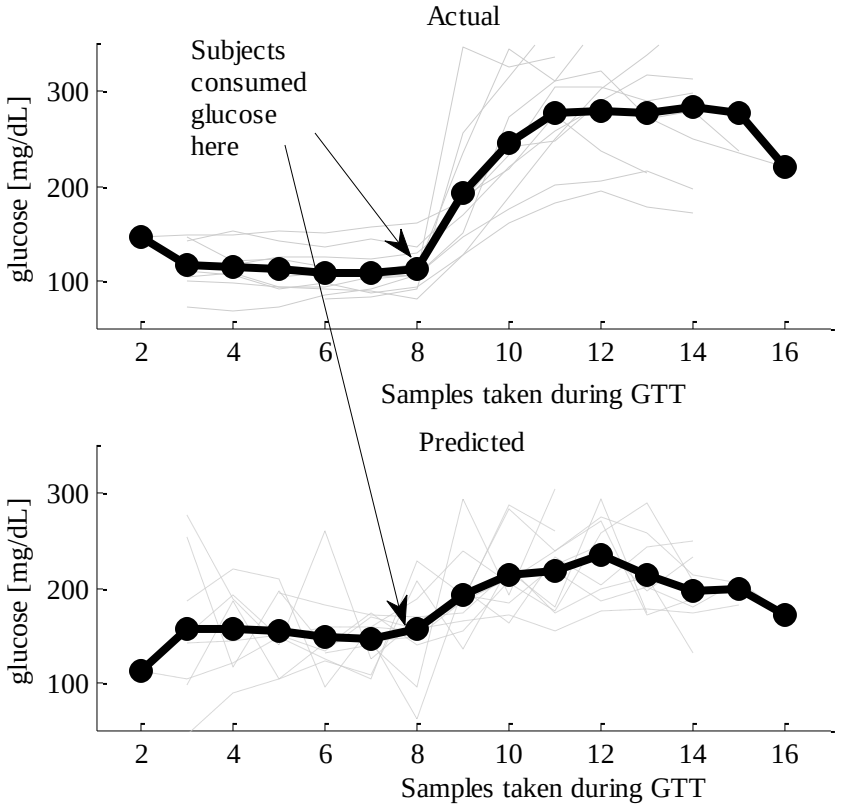

Figure 41: Prediction of continuous blood sugar levels using mixed effects model and leave-one-out cross validation. The top plot is the actual blood glucose level for 11 GTTs and the bottom plot is the predicted blood glucose. Gray lines are from individual GTTs, and the black lines are the means across all runs.

While prediction results shown above in Figure 41 are noisy, the mean of the predicted glucose curves have a similar shape to the mean of the actual data, which indicates that the MOC metrics were able to predict glucose on average with the data given.

Clarke error grid analysis was also done to determine how well continuous prediction performed. A Clarke Error Grid is a plot of the predicted vs. actual glucose data. Regions labeled as A, B, and C are considered clinically acceptable regions while regions D and E are not acceptable. To understand the justification for clinical relevance, consider a predicted reading that is 400 mg/dL, but the actual glucose level is 40 mg/dL (region E). The patient may take a shot of insulin in response to the high prediction and potentially die. Likewise, if a predicted reading is 40, but the actual glucose level is 400 mg/dL (also region E), the subject may consume

glucose and then suffer from extreme hyperglycemia and ketoacidosis (a lowering of the blood pH). Current off-the-shelf invasive continuous glucose monitoring technology typically requires 95% of data points to fall within the A and B regions of the Clarke Error Grid with 0% in the D and E regions while an acceptable mean absolute relative difference (MARD) is under 20%.

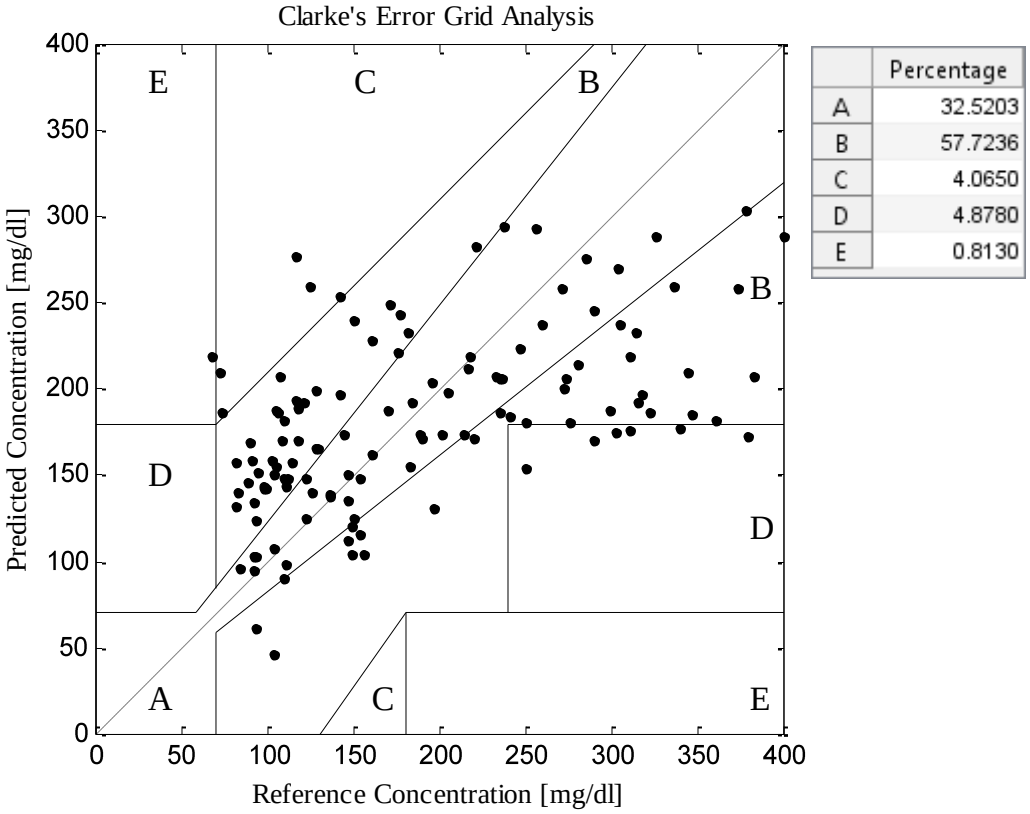

Figure 42: Clarke error grid analysis using leave-one-out cross-validated mixed effects continuous prediction of glucose using optimal predictors.

The results displayed in the Clarke error grid demonstrate continuous prediction using MOC inhibition metrics. The figure is a plot of predicted vs. actual glucose data which shows clinically relevant regions of the prediction accuracy; region A being the most accurate and also the most clinically benign region for an inaccurate estimation to occur and likewise, E being the least accurate and the most clinically dangerous region where an inaccurate estimation can occur. Parameters of the model fit are r^2=0.31 ($p<0.001$). As is shown in the Clarke error grid, 32.5% of the data points fall within the A region. While this percentage is low, it is also worth noting that

90% of the data points fall within the A and B region combined, indicating that, while the precision of the glucose estimate is lower than commercial continuous glucose sensors, the method does succeed in performing rough estimation of blood glucose, and with improvements to the methodology, this may eventually prove to be clinically useful Only one data point fell in the most clinically dangerous E region, and that point fell very near the border between E and C regions. A total of 5% of data points fell in the clinically dangerous D region.

It is useful to compare these metrics with those published in commercial enzyme-based invasive continuous glucose sensors built by the companies including industry leaders - Dexcom Inc. and Medtronic Inc.

Sensor	A+B	A	B	C	D	E	MARD
Dexcom SEVEN	97.8%	70.4%	27.5%	0.6%	1.5%	0%	16.7%
Medtronic RT CGMS	95.9%	74.4%	21.5%	0.6%	3.4%	0.1%	15.8%
MOC-based methods	90.2%	32.5%	57.7%	4.1%	4.9%	0.8%	37.2%

Table 6: Clarke error grid and mean absolute relative difference (MARD) comparisons between enzyme-based invasive glucose monitoring methods and the MOC-based methods described in this dissertation. Dexcom SEVEN results were published by Zisser et al. (2009) and Medtronic RT results were published by Mastrotoaro et al. (2008) .

While the MOC-based methods for estimating blood sugar are clearly not as accurate as current products used to estimate blood sugar using enzyme-based sensors, Table 6 above provides a comparison and enables a view of where the MOC-based methods would need to be improved if the method is to be adopted within a clinical setting.

2.6. Blood glucose within auditory model

In this chapter we have shown how SF OAE amplitude and MOC inhibition amplitudes change during hyperglycemia for a small sample of test subjects with diabetes. As shown in Figure 25, Figure 26, and Figure 27, the fraction of the SF OAE amplitude inhibited by MOC

activation increased during hyperglycemia. The amount of change measured for diabetic subjects is given in Table 7 below.

Metric	Normal glycemia	Hyperglycemia	Difference	p-value
Ipsilateral MOC inhibition	0.43	0.69	0.26	0.001
Contralateral MOC inhibition	0.26	0.42	0.16	0.022
Bilateral MOC inhibition	0.43	0.71	0.28	0.001
SF OAE Amplitude	15.6 dB SPL	18.0 dB SPL	0.27	0.049

Table 7: Change in MOC inhibition amplitude ($\Delta V_{MOC}(g)$) and SF OAE amplitude ($\Delta V_{SF\ OAE}(g)$) caused by glucose changes (g).

This change in MOC inhibition amplitude and SF OAE amplitude caused by hyperglycemia ($\Delta V_{MOC}(g)$ and $\Delta V_{SF\ OAE}(g)$, respectively) can be shown within the Ferry & Meddis basilar membrane model (2007) as indicated by the red dotted boxes in Figure 43 below.

DRNL filter bank with extensions including glucose effects on the MOC and SF OAE

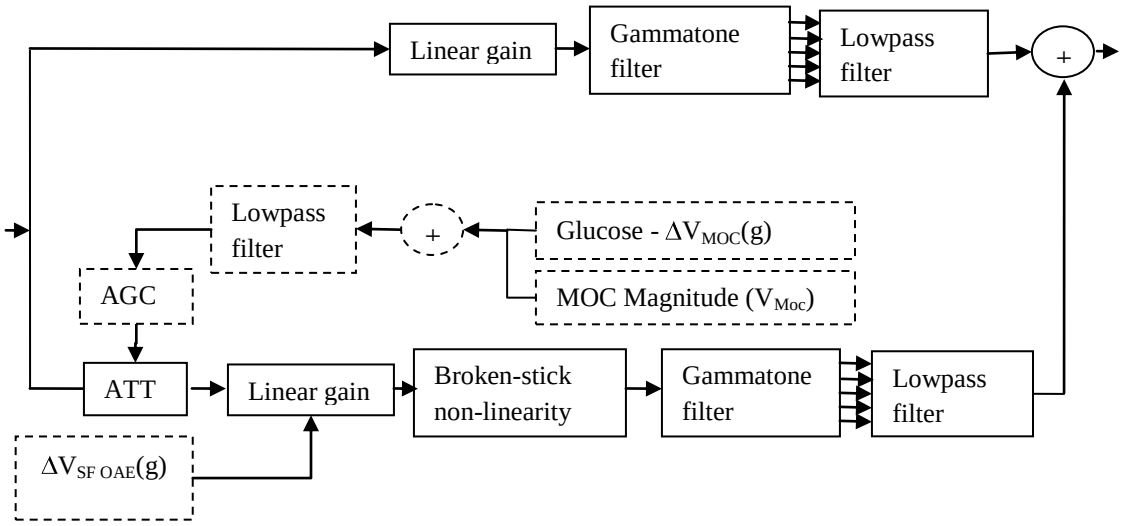

Figure 43: Extension of Ferry & Meddis model (2007) to include influence of blood glucose on both the MOC, $V_{MOC}(g)$, and on SF OAE amplitude $V_{MOC}(g)$.

We have also presented several statistical models that have enabled us to quantify the influence of blood glucose on MOC inhibition amplitude and phase as they are used within a prediction model

for glucose. These parameters are listed in Table 4 for predicting hyperglycemia (blood glucose > 160 mg/dL) and in Table 5 for predicting continuous blood glucose.

2.7. Discussion

Several important new findings have been presented within this research that can be summarized as follows:

- Results show that MOC inhibition mean amplitude consistently increased during hyperglycemia (glucose > 160 mg/dL) for all diabetic subjects tested ($p<0.05$).
- SF OAE amplitude in diabetic and non-diabetic subjects also increased during elevated sugar levels, but the increase, although statistically significant ($p<0.05$) for diabetics and non-diabetics was not consistent across all subjects tested.
- A new method has been presented called hyperglycemic risk analysis (HRA) that utilizes MOC inhibition amplitude and phase metrics as predictors to estimate hyperglycemia. ROC analysis shows an AUC of 0.85 when using mixed effects to predict hyperglycemia on cross-validated data.
- A method for predicting glucose using MOC inhibition amplitude and phase has been presented that enables classification of 90.2% of predicted glucose readings within the A and B regions of the Clarke error grid with total absolute relative difference of 37.2%
- A control experiment has been presented in which three diabetic subjects consumed water rather than sugar and water during the testing period. MOC inhibition amplitudes were not statistically different than MOC inhibition amplitudes acquired on a separate day during normal-glycemia.
- Results from non-diabetic subjects, tested using higher level elicitors that evoked both the MOC and the MEMR, show a comparable mean increase in MOC inhibition amplitude

when glucose levels rose above 115 mg/dL, but this increase was not statistically significant

- There was no observed effect of hyperglycemia on MOC or SF OAE timing metrics including onset rise-time and latency.

The original hypothesis for this study was that the MOC and SF OAE amplitude and timing metrics would correlate with blood sugar levels. While timing metrics (latency and rise-time) did not show a correlation with blood glucose, MOC inhibition amplitude and SF OAE amplitude both correlated with blood glucose based on group data as analyzed using t-test analysis and cross-validated mixed and non-mixed effects model fitting analysis. Based on prior work that showed cochlear microphonic and endolymphatic potential declining during hypoglycemia (Mendelsohn & Roderique, 1972; Wing, 1959; Koide, 1958) and DP OAE amplitudes also declining during hypoglycemia (Zuma e Maia & Lavinsky, 2006; Zuma e Maia F. , Lavinsky, Mollerke, Duarte, Pereira, & Maia, 2008), we expected the SF OAE amplitude to have a positive correlation with blood glucose and thereby increase with blood sugar levels. Results in both diabetic and non-diabetic subjects showed a statistically significant ($p=0.049$ for diabetics, $p=0.028$ for non-diabetics) increase in SF OAE amplitude during elevated sugar levels. While our results are in agreement with Zuma e Maia et al., they conflict with prior work done by Suckfull et al. (Suckfull, Winkler, Thein, Raab, Schorn, & Mees, 1999) in which they observed DP OAEs to be reduced in amplitude during increased glucose perfusion of the cochlea in rabbits. Suckfull believed that changes in DP OAE amplitude in his experiments were likely due to changes in serum osmolarity, not glucose elevation per se. Serum osmolarity had been shown to change outer hair cell function (Chertoff & Brownell, 1994). However, it is also possible that Suckfull's perfusion of the rabbit's cochlea with glucose solution was affecting primarily the second mechanism described in section 2.1.2.2 whereby ATP acts not only as an energy source for the cochlea (thereby leading to changes in the EP and cochlear microphonics – see section

2.1.2.1), but also as a neurotransmitter. In this way Suckfull's work would parallel that of Kujawa et al. (1994a) in which DP OAE amplitudes were reduced by perfusion of the perilymph with ATP solution. Activation of ATP-sensitive ion channels cause depolarization of outer hair cells, which causes them to be less sensitive to acoustical signals and reduces the gain of the cochlear amplifier (Yu & Hong-Bo, 2008), which would then lead to smaller DP OAE amplitudes. Kujawa proved that reductions in DP OAE amplitudes were due to ATP-sensitive ion channels by presenting ATP antagonists and showing that the effects could be abolished (1994b). The fact that there are two mechanisms that could be impacting OAE amplitudes during hyperglycemia, and that these two mechanisms act in opposing directions on the outer hair cells, could potentially explain how the body maintains consistent cochlear function during glucose changes. For example, when glucose levels are elevated, the EP and cochlear microphonics become elevated which makes the cochlea more sensitive to sound; however, the ATP-sensitive ion channels also become activated which causes a reduction in the gain of the cochlear amplifier, thereby reducing the sensitivity of the cochlea to sound. Perhaps these two opposing mechanisms cancel each other out. This could explain why Sasso et al. (1999) showed no change in DP OAE amplitudes during a hyperglycemia clamp experiment in 10 diabetic and 10 control subjects. And it could also explain why several of the individual subjects tested in the research presented here and prior work done by us (Jacobs, Wan, & Konrad-Martin, 2008) did not demonstrate a statistically significant change in SF OAE amplitude during the GTT.

If there are indeed two mechanisms that cause glucose-related changes to the cochlea but in opposite directions, the MOC perhaps provides the tie-breaker by mediating and enhancing the second mechanism in which ATP is acting as a neurotransmitter. Recall that ATP-related effects on ion channels cannot happen without the presence of calcium ions (Ashmore & Ohmori, 1990; Nakagawa, Akaike, Kimitsuki, Komune, & Arima, 1990; Dulon, Mollard, & Aran, 1991; Housley, et al., 1999; Yu & Hong-Bo, 2008). And it is the activation of the MOC which enables

release of this calcium. When the MOC is activated, there is a release of the neurotransmitter acetylcholine (Frolenkov, Mammano, Belyantseva, Coling, & Kachar, 2000; Blanchet, Erostegui, Sugasawa, & Dulon, 1996; Evans, 1996) which has been shown to lead to release of extracellular calcium ions (Elgoyhen, Vetter, Katz, Rothlin, Heinemann, & Boulter, 2001; Guinan Jr. J. J., 2010). Since ATP increases in the endolymph during a hyperglycemic event as shown by Puschner and Schacht (1997), and calcium ions increase when the MOC is activated as discussed above, we would therefore expect to see a larger MOC inhibition amplitude during hyperglycemia than during normal glycemia. This could be the explanation for why we observed an increase in MOC inhibition amplitude during hyperglycemia in all diabetic subjects for all MOC elicitor methods tested as shown in Figure 25-Figure 27.

Results from this study also demonstrate that to observe the affect of glucose on the MOC, it is important to activate only the MOC, and not the MEMR. Results on the non-diabetic subjects were obtained using higher level MOC elicitors that were simultaneously eliciting MEMR. This was clearly the case because the inhibitive effect of the elicitors were larger than the amplitude of the SF OAE, which indicates that the input impedance of the ear canal was being increased through the activation of the stapedius muscle, causing reduction in amplitude of the total pressure in the ear. The change in pressure due to the MEMR was significantly more variable than the change due to MOC activation, and this was probably partially why no statistically significant influence of blood sugar on the MOC+MEMR was observed for the non-diabetic subjects. However, it is interesting to note that when the mean change in acoustical inhibition during blood sugar elevation was compared between normal and diabetic subjects, the mean change was very close to the same for all elicitor types as shown in Figure 30. Also shown in Figure 30 is that the group mean change in inhibition was systematically (across all three elicitor types) smaller for the non-diabetic subjects, which is what we would expect since the nondiabetic subjects had a smaller change in blood glucose levels than the diabetic subjects.

Nondiabetic subjects also demonstrated a smaller increase in SF OAE amplitude during elevated blood sugar levels than did the diabetic subjects as shown in Figure 30. The smaller glucose related change in MOC and SF OAE amplitudes in nondiabetic subjects is what is expected since the cut-off for "hyperglycemia" for the nondiabetic group was 115 mg/dL while it was 160 mg/dL for the diabetic group.

The control experiment on three nondiabetic subjects was critical for this study; it showed that time-related changes in SF OAE amplitude and MOC amplitude were not confounding results. MOC amplitudes recorded during hyperglycemia were significantly different than those obtained during the control experiment, in which subjects consumed only water during the 4-hour test period.

Results from prediction models demonstrated that hyperglycemia in diabetic subjects (glucose >160 mg/dL) could be predicted with an accuracy of 83% and a false-positive rate of 23% depending on where the cut-off in classification is set as shown in the cross-validated leave-one-out ROC curves and histograms in Figure 39 and Figure 40. Use of a mixed effects model significantly improved prediction accuracy and reduced false-positives. The random subject-specific effect that was integrated out of the model was the mean MOC inhibition amplitude. Notice from Figure 25, Figure 26, and Figure 27 that the normal-glycemia MOC inhibition amplitude varies significantly between the test subjects. The mixed effects model accounts for this variability and removes this random effect from the prediction model. However, it presumes that we know ahead of time what the individual subject's normal glycemia MOC inhibition amplitude is. In the prediction analysis, this was known because subjects underwent two GTTs each. Therefore, if one subject's GTT was left out of a model training session, their random effect normal-glycemia MOC amplitude could still be estimated using their other GTT run, and used within the prediction. Changes during hyperglycemia were not considered as random effects, only as fixed effects. In this way, it was assumed that all subjects' blood glucose would

change in a similar amount. This enabled us to create a fixed effects prediction model as shown in Table 4 that is used to estimate the general group effect glucose has on each of the predictors.

While we were able to predict continuous glucose levels using the optimal metrics found within HRA as shown in Figure 41 and Figure 42, prediction accuracy was not comparable with off-the-shelf continuous glucose monitors (see Table 6). Specifically, while 90.2% of the predicted data points fall within the A+B region of the Clarke error grid, only 32.5% fall in the A region, suggesting that prediction accuracy was certainly not as accurate as other invasive methods of glucose estimation based on enzyme sensors. This suggests that there could be room for improvement in the methods described. This could be a rich area for future research.

One area of future research would be in the area of improved methods for extracting the MOC. While overall, the MOC inhibition amplitudes showed reasonably good repeatability for the subjects in test/re-test in that means remained statistically the same, the variability was still high. If variability of MOC recording could be reduced, this could enable more accurate prediction using MOC inhibition as the metric. One way for improving the accuracy could be to record MOC inhibition across the entire basilar membrane using a chirp as the SF OAE elicitor while still using noise as the MOC elicitor, and then taking the SF OAE-normalized mean MOC inhibition amplitude across all of the frequencies. Choi et al. (2008) described a method whereby a stimulus frequency was swept from 550-1450 Hz to evoke an SF OAE. If this method of chirp-based SF OAE elicitation could be combined with an MOC elicitor, it could potentially improve the accuracy of MOC inhibition amplitude estimates which could ultimately improve accuracy of glucose predictions using MOC as the predictor. It would also be interesting to observe how glucose affects different regions of the basilar membrane, and this information would also become known through the use of a swept stimulus frequency.

Another area of future work could potentially be in the area of isolating the two mechanisms that glucose has on cochlear function; (1) as an energy source and regulator of endocochlear potential and (2) as a neurotransmitter. While isolation of these two mechanisms in humans would be difficult, it may be possible within an animal model. Consider an experiment in which one would simultaneously monitor and control (through the administration of furosemide) the endochlear potential during an experiment in which ATP is simultaneously delivered to the cochlea. Various ATP agonists and antagonists could be used to observe whether the ATP-based effects are acting on the ATP-sensitive ion channels and thereby causing inhibition of microphonics and OAEs while the EP remains constant.

Chapter 3

3. Focused auditory attention effects on the efferent pathway

In the previous chapter, we demonstrated that the MOC complex amplitude increased in diabetic subjects during hyperglycemia. In this chapter, we present results from an experiment in which we investigated the impact of cortical influences on the MOC complex amplitude. The glucose tolerance tests (GTT) described in chapter 2 typically required 4 hours to complete. Because of prior literature suggesting that the MOC can change based on input from the auditory and visual cortex (Mulders & Robertson, 2002; Hernandez-Peon, 1966; Hernandez-Peon, Scherrer, & Jouvet, 1956; Perrot, et al., 2006; Maison, Christophe, & Collet, 2001; Harkrider & Bowers, 2009; de Boer & Thornton, 2007)., there was a question regarding whether the test subject's auditory attention may be changing during the GTT and possibly causing anomalies in the results. We implemented strict control over the diabetes subjects' attention during the GTTs. We required diabetic subjects to watch movies with subtitles during the course of the glucose tolerance testing, thereby providing some control over their attention. However, non-diabetic subjects were tested in an earlier experimental paradigm in which subjects' attention was not strictly controlled; some of the subjects watched the silent movies, while some of them did not. In this way there was a question regarding whether the subjects might be paying closer attention to the auditory signals at different times during the experiment, and thereby biasing the results. In this chapter we show results from a test in which we evaluated whether there was a difference in MOC inhibition amplitude during an active listening task compared with a passive listening task. During the active listening task, the SF OAE was evoked in the ipsilateral ear and the MOC was elicited in the contralateral ear using broadband noise as a stimulus. Within the broadband noise, subjects were asked to identify the presence of a tone in a two-alternative forced choice paradigm. During the passive listening task, the same audio stimuli were presented, but the subjects received no instructions other than to sit and watch a silent movie during the testing. The purpose was to

determine whether subjects' attention caused changes in the MOC inhibition amplitude. A secondary test was included within the experimental paradigm in which different frequency cues were presented immediately prior to the target tone stimuli in the contralateral ear. Cues were either at the same frequency as the target tones, at a frequency outside of the critical band, or else not present at all. A secondary objective of this experiment was to investigate whether the subjects' MOC inhibition amplitudes changed relative to the cue frequency and also relative to the signal-to-noise ratio of the target probe within the noise elicitor. There was prior work indicating that a subjects' attention bandwidth – the frequency range over which they are searching for a stimulus – is dependent on the functionality of the MOC (Scharf B. , Magnan, Collet, Ulmer, Ulmer, & Chays, 1994; Scharf, Magnan, & Chays, 1997).

3.1. Background

The MOC starts at the superior olivary complex (SOC) and extends to synapse on the cochlear basilar membrane, and is hypothesized to be the descending efferent pathway that could play a role in this early stage selective attention. Early work on the role of attention in efferent nerve activation was done by Hernandez-Peon when in 1966 he proposed a filter model whereby selective attention activates efferent fibers that enables early stage auditory discrimination of target auditory signals from noise (Hernandez-Peon, 1966). This theory was based partly on cat experiments in which changes in electrical activity in the cochlear nucleus were observed during an attention experiment (Hernandez-Peon, Scherrer, & Jouvet, 1956). More recently, Mulders and Robertson (2002) and Spangler and Warr (1991) have shown from anatomical experiments that a pathway exists between the auditory cortex and the SOC both directly and via the inferior colliculus. Mulders and Robertson (2002) demonstrated that direct electrical stimulation of a guinea pigs inferior colliculus resulted in a significant reduction in amplitude of the compound action potential in the auditory nerve in response to auditory tone stimuli. The suppression due to IC stimulation was equivalent to 3-5 dB of equivalent amplitude gain reduction. Human

studies have further suggested that there may be a "top-down" component to control of the MOC. Perrot et al. (2006) demonstrated that stimulating the auditory cortex of epileptic patients during surgery resulted in significant decrease in evoked OAE amplitude being measured during the stimulation.

Less invasive procedures further suggest that there is a top-down control mechanism that is used to control the MOC. The effect of listening task (active vs. passive) on MOC inhibition of OAEs has been studied by several researchers (Maison, Christophe, & Collet, 2001; de Boer & Thornton, 2007; Harkrider & Bowers, 2009; Ferber-Viart, Duclaux, Collet, & Guyonnard, 1995). Maison et al. (2001) recorded evoked OAEs in the ipsilateral ear at two test frequencies, 1 and 2 kHz. While OAEs were being recorded, the subjects were required to detect probe tones in background noise (which simultaneously elicited the MOC) in the contralateral ear. They found that the MOC inhibition effect on the OAEs increased at the frequency on which the subject's attention was focused. However, Harkrider and Bowers (2009) found that when subjects attended to a broadband noise MOC elicitor signal, the effect of contralateral suppression of click-evoked OAEs was reduced compared with when subjects were passively listening. They described this effect as resulting from a "cortically mediated release from inhibition". De Boer and Thornton (2007) found similar results as Harkrider and Bowers such that during an auditory attention task MOC inhibition increased as compared with the passive listening condition. It is important to address the differences in these results and to propose an explanation regarding the different outcomes. In this dissertation, we present results that are similar to Maison et al.'s results and present an explanation for why results across experiments may differ.

In addition to the auditory cortex, control of the auditory periphery may also arise from descending pathways originating at the visual cortex. Several studies have shown that hair cell motion as measured by evoked potentials and OAEs is reduced during visual tasks. Oatman (1976) showed that cochlear potentials in response to sound clicks were reduced at the auditory

nerve, cochlear nucleus, and auditory cortex as a result of visual stimuli. Meric and Collet (1992) observed a small but statistically significant effect of visual attention tasks on evoked OAEs. Meric and Collet found an equivalent reduction in OAE amplitude of 0.35 dB during presentation of visual stimuli to test subjects. However, as has been the case with auditory attention experiments, results with visual attention have not all been consistent. For example, Froehlich et al. (1990) observed a significant variability in reduction of evoked (e)OAEs due to visual attention tasks. Of 16 subjects, only three showed a significant reduction in eOAE amplitude during visual stimuli. However these three subjects were re-tested and the effect was repeatable. Other related studies have also shown that visual attention tasks reduce TE OAE amplitudes (Froehlich P. , Collet, Chanal, & Morgon, 1990; Froehlich P. L., Collet, Redmond, Jaboulay, Ferber, & Morgon, 1994; Puel, Bonfils, & Pujol, 1988) and a similar reduction in amplitude has been demonstrated in DP OAEs during visual stimulation (Ibarguen, Montoya, Rey, & Ana, 2008).

Evoked responses including auditory brainstem responses (ABRs) have also been shown to change in response to attention effects. Electrophysiology experiments on auditory brainstem responses have shown that wave I (auditory nerve) ABR responses to non-relevant auditory stimuli were lower in amplitude and increased in latency during the presence of visual stimulation (Lukas J. H., 1980; Lukas, 1981). Possible mechanisms for how attention can mediate the cochlear efferent response have been discussed and are summarized in Meric and Collet (1994).

Since the MOC has been shown to increase as well as decrease during auditory attention tasks, the question continues to be how the MOC improves listening ability in humans, and is this functionality cortically controlled or primarily a lower-brainstem reflex mechanism. A natural hypothesis is that the MOC will increase and decrease as required to improve listening capability (Guinan Jr. J. J., 2010). Before exploring this further, we must first understand the role of the MOC within the auditory system. The leading theory regarding the role of the MOC is that it is

used to improve detection of relevant target audio stimuli such as speech within a noisy environment. It is well known that noise elicits MOC activity as covered in depth in Guinan's review (Guinan Jr. J. , 2006). A study by Kumar and Vanaja (Kumar & Vanaja, 2004) showed that speech discrimination performance improved in the presence of noise for signal-to-noise ratios of +10 dB and +15 dB relative to speech discrimination in a quiet environment. Work by de Boer and Thornton (2008) has shown that efferent activity can predict and reflect improvement in speech-in-noise discrimination tasks. They further showed that MOC activity is adaptable and can change with training. Scharf et al. did a single-subject case study (1994) and later a 16-subject follow-up case study (1997) that demonstrated the role of the MOC in suppressing unexpected auditory stimuli. In this study, Scharf tested subjects suffering from Meniere's disease both before they underwent a vestibular neurotomy in which the olivocochlear bundle (OCB) was severed and afterward. While most audiometric functioning remained constant before and after the surgery for these subjects, nearly all of them demonstrated an improved ability to detect unexpected tones in noise after the surgery. Subjects were given a task in which they needed to guess the presence or non-presence of a target signal in noise. Immediately prior to the detection task, subjects were given an auditory cue that was either the same frequency as the target or at a different frequency. Prior work has shown that people with normal auditory functionality perform more poorly on this detection task when the cue is at a frequency outside of the critical band of the target (Tan, Robertson, & Hammond, 2008; Schlauch & Hafter, 1991). However, Scharf showed that this selective "attention bandwidth" disappeared in most of the subjects who had the OCB severed. He showed that subjects with the MOC severed performed equally well when given a cue with the same frequency as the target tone as when the cue frequency was outside the critical band of the target tone. This evidence, although not repeated by anyone yet, strongly suggests that cortical control of the auditory periphery may be used to discriminate target auditory signals such as speech from non-relevant noise.

3.1.1. Quantizing auditory attention using probe-signal methods

Detection of a tone within noise has been shown to be linked with the "critical band" (Scharf, Critical Bands, 1970), which is defined by its center frequency and a bandwidth determined by physical perception experiments. Fletcher (1940) showed that a tone presented in noise remains detectable at a constant noise threshold so long as the bandwidth of the noise is larger than the critical bandwidth about the signal center frequency. Once the bandwidth of the noise is reduced lower than this critical bandwidth, the threshold of detection increases as the bandwidth decreases. A question asked by Schlauch and Hafter (1991) was whether these bandwidths could be effectively widened by introducing the concept of uncertainty within the signal frequency that was to be detected. They found that indeed these listening bands grew from 12% of the center frequency to about 15% when the uncertainty about the signal being detected increased. They showed that focused auditory bandwidths changed depending on the frequency of the auditory stimuli on which the subject's attention was focused. In Schlauch and Hafter's experiment, subjects were asked to identify whether a target tone was present in noise. Immediately prior to presentation of the noise and target probe, a cue was presented. The cue was a tone that was either the identical frequency as the target probe, or alternatively a tone distant in frequency from the target. Subjects were asked if they heard the target tone within the noise under different cue frequency conditions. If the subject correctly identified the interval in which the tone was presented in the noise, that question was scored as correct. They showed that the percent correct for subjects increased when the cue tone was closer in frequency to the target tone. Subjects had to broaden their auditory attention bandwidth when the cue was further away from the target frequency. When subjects had to search a broader frequency range, the task became more difficult and their accuracy declined. This can be alternatively described as subjects using a small Q attentional filter – broadly tuned and lower in amplitude. Subjects

focused their attention narrowly when the cue frequency was near or identical to the frequency of the target and their percent correct scores increased. This can be alternatively described as subjects using a large Q attention filter – narrowly tuned and higher in amplitude.

In the attention experiment described in this chapter, we first examined whether the MOC inhibition changed during active vs. passive probe-finding tasks. Next, we attempted to understand whether changes in MOC inhibition were affecting improvement in subjects' performance during the different cue conditions. We used the probe-detection scheme described by Schlauch and Hafter (1991) and extended it by measuring the MOC inhibition amplitude as it might change with respect to the bandwidth of the subject's attention; the bandwidth of the subject's attention was changed by changing the frequency of the cue that precedes the target probe signal. More specifically, we evoked SF OAEs in the ipsilateral ear and presented target tones in the contralateral ear while the MOC was elicited using contralateral noise. Cues were used to help the subject identify the target tones within the contralateral noise. The cues were either on-frequency (same as the target probe), off-frequency (outside of the critical band of the target probe), or no cue at all. We investigated changes in MOC inhibition of the SF OAE during active vs. passive listening tasks, using different frequency cues, and also looked at changes in MOC inhibition amplitude with different signal-to-noise ratios of probes.

3.1.2. Hypothesis of active vs. passive influences on MOC

Our hypotheses for the outcomes are based on the theory that MOC inhibition should increase if an increase leads to improvement in performance at a given task and similarly, MOC inhibition should decrease when a decrease in MOC inhibition leads to improvement in performance at the task. Since the SF OAE elicitor tone is present in the ipsilateral ear, but the target tones that subjects are asked to identify are in the contralateral ear, the ipsilateral tones are likely to be considered as distracting signals to the listeners, and we would expect that inhibition of these tones caused by activation of the MOC would improve performance. Therefore we

expect larger MOC inhibition of SF OAE amplitudes during the active listening task as compared with the passive listening task.

3.1.3. Hypothesis of cue influences on MOC inhibition

The hypothesis for MOC activation correlations with the cue type are based primarily on the Scharf et al. experiments which demonstrated how subjects with severed MOC performed equally well in probe-detection experiments when given a cue within the critical band vs. outside of the critical band (Scharf B. , Magnan, Collet, Ulmer, Ulmer, & Chays, 1994; Scharf, Magnan, & Chays, 1997). Given Scharf's results, we expect that a cue tone acts like a spotlight on a critical bandwidth on the basilar membrane – activating the MOC and causing inhibition everywhere outside of the critical band of the cue tone frequency. So if a cue tone is presented at the same frequency as the target, we would expect that the MOC would inhibit all other frequencies outside of this critical band, but that the probe tone would not be inhibited as shown in Figure 44. This would improve performance of a signal detection task when the target is within the critical band of the cue.

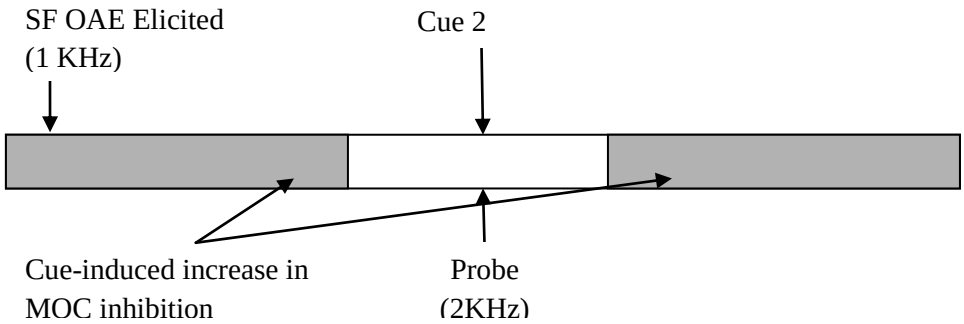

Figure 44: Demonstration of proposed inhibition paradigm during probe detection with a cue presented prior to the probe. In this paradigm, the cue is presented immediately prior to the probe and at the same frequency as the probe the probe. We would expect that the probe would not get further inhibited through activation of the MOC. We would also expect that the MOC inhibition would be larger due to the cue activation. This theory is based on work by Scharf et al. (1994; 1997).

Following this same logic, performance would be worse when the target is outside of the critical band of the cue, since we are theorizing that inhibition will be larger outside of the cue's critical band.

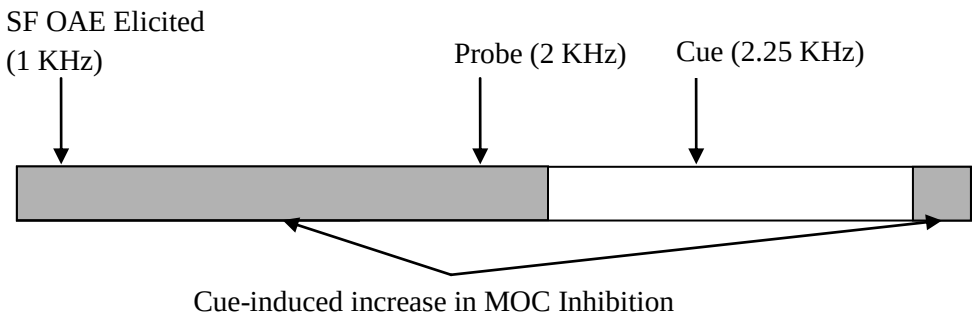

Figure 45: When the cue is at a frequency outside of the critical band of the probe, it becomes more difficult to detect the probe in noise (Schlauch & Hafter, 1991). This may be because the cue activation causes increased MOC inhibition in the region outside the cue.

The improvement in performance observed when the cue and probe are at the same frequency may be caused by either (1) increased MOC inhibition outside of the cue's critical band or alternatively (2) a release from MOC inhibition inside of the critical band of the cue. If hypothesis (1) above is correct, we would expect to see a larger MOC inhibition amplitude during cue presentation. If hypothesis (2) above is correct, we would not expect to see a change in MOC inhibition amplitude based on cue, because the improvement in performance is due to a release from MOC inhibition within the cue's critical band, not due to an increase in MOC inhibition outside of the cue's critical band.

3.2. Methods

A total of ten test subjects were evaluated in this experiment. Each subject was screened in a 2-hour test to determine whether they exhibited normal hearing, a robust MOC inhibition, robust SF OAE amplitudes, and a high threshold for middle ear muscle reflex. SF OAEs were evoked in the ipsilateral ear while the MOC was elicited in the contralateral ear using broadband noise. Target tones were presented to the subject's contralateral ear during the noise presentation

and subjects were asked to identify the presence of these targets in the noise. Immediately prior to presentation of the target tone, a cue was presented to the subject's contralateral ear either at the same frequency as the target probe or at a frequency outside of the critical band of the probe. There was also a condition whereby no cue was presented. Performance of the subject on the probe identification task was done by recording the total percent correct identification of the probe in noise in a 2-alternative forced choice testing paradigm. Prior to performing the above test, the individual subject's signal-to-noise ratio at which they were achieving nearly 100% correct in the on-frequency cue condition was found. We investigated (1) correlations of MOC inhibition amplitude with active vs. passive listening, (2) correlations of MOC inhibition amplitude and cue type, and (3) correlation of MOC inhibition amplitude on probe signal-to-noise ratio.

3.2.1. Screening test

During the screening visit, subjects were screened for robust MOC, middle ear activation at different elicitor levels, and robust SF OAEs. The subjects' SF OAE was elicited through the presentation of a 12-second pure tone at each of four probe frequencies: 1000 Hz, 1025 Hz, 1050 Hz, and 1075 Hz. The probe was presented at 55 dB SPL and the level was confirmed through automated calibration in a B&K 4157 artificial ear and the custom-built data acquisition system described in section 2.2.5. The MOC inhibition was elicited ipsilaterally, contralaterally, and bilaterally with broadband noise at a level of 60 or 65 dB SPL (depending on subject's MEMR threshold) applying the same methods described in section 2.2.5.

SF OAEs were evoked using probe frequencies (f_{probe}) of 1000, 1025, 1050, and 1075 Hz and suppressor frequencies at $0.95*f_{probe}$ (950 Hz, 974, 998, and 1021 Hz). Probe levels for the SF OAE and MOC elicitation were presented at 55 dB SPL and the SF OAE suppressor probe level was presented at 75 dB SPL to ensure that the SF OAE evoked by the probe was being fully suppressed by the suppressor tone.

During this screening a middle ear muscle reflex was also performed using a 602 Hz probe tone and a broadband noise elicitor at 55, 60, 65, and 75 dB SPL. Amplitude and phase responses to the probe tone were recorded both during the noise elicitor and without it present. If a measurable amplitude or phase change occurred during the noise elicitor, then the MEMR was declared to be present at that elicitor level. In subsequent experimentation, elicitor levels were only used if they did not elicit a middle ear muscle response.

Subjects passed the screening if they satisfied each of the three criteria listed below:

(1) Robust MOC at one of the four probe frequencies

(2) Robust SF OAE at one of four probe / suppressor combinations

(3) No middle ear muscle reflex at 65 dB SPL or below

3.2.2. Attention tests

The attention tests described here extend the early psychophysical experiments done by Schlauch and Hafter (1991) in that both attention bandwidth and MOC activation magnitude were measured simultaneously so that the effect of auditory attention on MOC amplitudes could be measured. In this experiment, two tests were done, a "Find Detection Threshold Test" and an "Attention Correlation Test". The "Find Detection Threshold Test" was used to determine the appropriate probe levels ultimately used within the "Attention Correlation Test". And the "Attention Correlation Test" was used to determine the effect that different cue frequencies have on MOC inhibition amplitudes both during passive listening and during active listening; when the subject was asked to pay attention to a sound and identify it within noise.

3.2.3. Find detection threshold test

In the "Find Detection Threshold Experiment", the objective was to determine at what minimum probe level the subject could detect, at nearly 100% accuracy, whether a probe tone was present during a noise sound presentation when an on-frequency cue was used to aid the

subject in detection. A single sound presentation/recording for this test lasted 4 seconds as is shown in the timing diagram below. The presentation window starts at 0 seconds and 0.4 seconds after the start of the presentation window, a 200 ms pure tone cue was presented to the subject's contralateral ear. An OAE elicitor pure tone at 1000 Hz, 1025 Hz, 1050 Hz, or 1075 Hz (depending on the subject's largest SF OAE amplitudes) was presented starting one second after the start of the presentation window to the subject's ipsilateral ear. This OAE elicitor was presented for 3 seconds and extended through the end of the presentation window. Between seconds 1-2 after the start of the presentation window, a noise elicitor was presented to the subject's contralateral ear. Embedded within this contralateral noise, 7 dB lower than the cue level, was either a probe tone or else nothing. The subject was trying to guess whether this probe tone was present within the noise or not. See the timing diagram below for a complete visual display of the presentation.

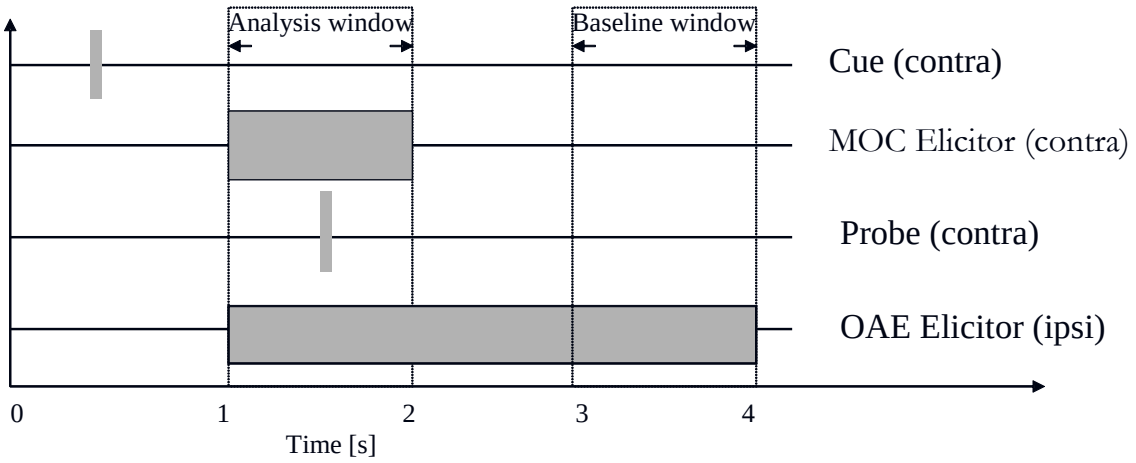

Figure 46: Timing diagram for attention experiment. The above was presented twice to the subject in a two-alternative forced-choice experiment. In one randomly selected presentation, the probe was presented, and in the other presentation, the probe was not presented. The subject had to guess in which presentation the probe was present.

Notice in the timing diagram that the time between seconds 1 and 2 is termed the analysis window. The analysis window was the time during-which the noise elicitor was active in the contralateral ear. An FFT of the measured response at the OAE elicitor frequency in the

ipsilateral ear was done both during the analysis window when the MOC was activated and during the baseline window when the MOC was not active. The vector subtracted difference between the baseline window magnitude and the analysis window magnitude was the MOC complex amplitude as is described in further detail in section 2.2.6.1

A two-alternative forced choice (Levitt, 1971) 5-down, 1-up test paradigm was used to determine the minimum probe level at which the subject could achieve nearly 100% accuracy. Probe levels were presented first at levels very loud (70 dB SPL) and easily detectable within the noise. After the subject guessed correctly five consecutive times, the probe level was reduced by 10 dB. If the subject answered correctly another five consecutive times, the probe level would decrease again by 10 dB. This would continue until the subject answered one incorrectly, at which point the probe level would increase. The amount of change in the probe level resulting from the subject's response was reduced adaptively by half its value every time a reversal occurred. A reversal was defined as either (1) getting at least five consecutive correct and then one incorrect, or (2) getting one incorrect, and then getting at least five consecutive correct. The minimum amount of change in probe amplitude was 1 dB.

A total of 200 presentations were given to each subject to evaluate their minimum threshold at which they achieved nearly 100% accuracy. An average of the probe level across the final 20 presentations was used to estimate the subject's minimum detection probe level. This minimum detection probe level, increased by one decibel, was used in the MOC Attention Experiment. The reason why we increased by one additional decibel is because we were finding that if we used the minimum detection probe level as found using an on-frequency cue, most subjects could not detect the probe when no cue was given. The subjects were just guessing during the no-cue condition. We felt it was important for subjects to not be guessing in the no-cue condition, as their attention might begin to drift during the experiment, thereby altering the results. By raising the minimum detection probe level one dB above that found during the "Find

Detection Threshold Test", it was still challenging for the subjects to detect the probe in the no-cue condition, but they were no longer guessing.

Shown below is an example of one subject's Find Detection Threshold Test results and how the probe level adaptively moves to the final detection threshold.

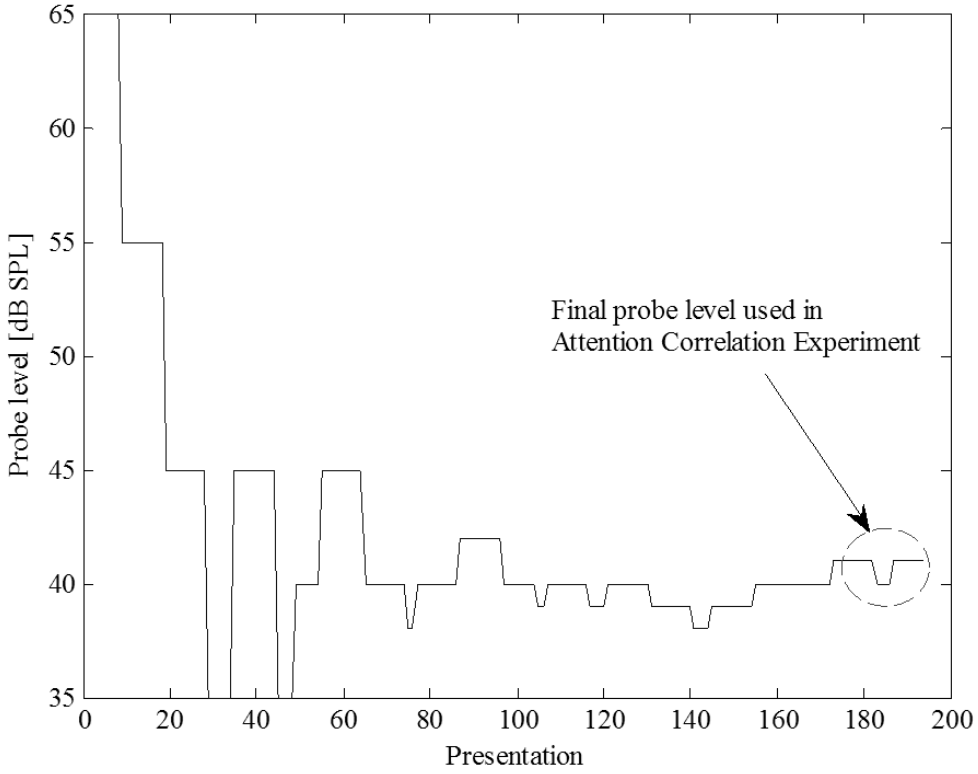

Figure 47: Find Detection Threshold Test results for one subject. Notice that when the subject answered 5 in a row correctly, the probe level dropped adaptively. If the subject answered one incorrectly, the probe level would increase adaptively. The change in probe level was large (10 dB) at the beginning of the experiment and by the end the change was only 1 dB.

3.2.4. MOC attention correlation test

The purpose of the MOC Attention Correlation Test was to determine whether MOC amplitude varied with the subject's auditory attention to different frequency sound cues. The same presentation paradigm was used as that described under the Find Detection Threshold Test in the previous section with several exceptions. First, the probe level was presented only at the

minimum probe level determined by the Find Detection Threshold Test. Second, the cues used during the presentation were not always the same frequency as the probe. Specifically, the purpose of this test was to determine whether the MOC amplitude varied depending on whether the subject was presented with (1) a cue with the identical frequency as the probe being searched for by the subject, (2) a cue with a different frequency than the probe frequency, or (3) no cue at all. The probe frequency was always 2 KHz and the cue was either 2 KHz (on-frequency condition), 2.25 KHz (off-frequency condition), or no cue at all (no cue condition). The frequency of the cue for the off-frequency condition cue was selected to be 2.25 KHz since this frequency was outside of the critical band of the 2 KHz probe frequency.

3.3. Results

In this section, results will first be presented from the behavior testing, demonstrating that the three cue conditions did lead to changes in performance accuracy. Results will be presented from the Find Detection Threshold Test and we will show how MOC inhibition complex amplitude did not change relative to the signal-to-noise ratio during this test. The primary discovery during this study will then be presented; which is that MOC inhibition amplitude tended to increase during active attention listening tasks as compared with passive listening tasks. And finally results of MOC inhibition during the three different cueing types will be presented.

3.3.1. Results of behavior during testing

For all subjects, we were able to find the detection threshold at which they were just barely achieving 100% correct. The influence of the three different cue paradigms- no cue, off-frequency cue, and on-frequency cue – yielded behavioral results very close to what we would have predicted. Figure 48 shows how overall (demonstrated by the mean across subjects), people performed the best in the on-frequency condition and worst on the no-cue condition.

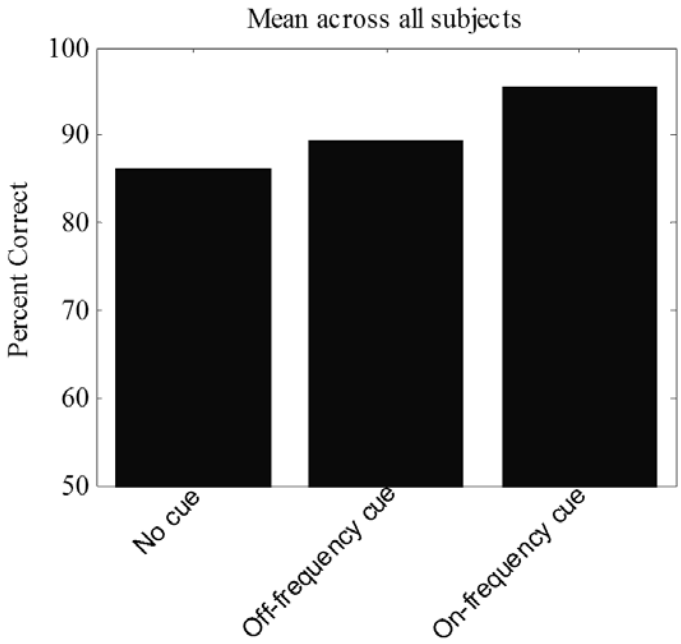

Figure 48: Mean percent correct across all 10 subjects.

However, not all of the subjects followed the pattern shown in Figure 48. Shown below in Figure 49, while subject 62 followed the trend of performing least well on the no-cue condition, followed by the off-frequency cue, and finally performing best in the on-frequency cue condition, some subjects, such as subject 43 performed equally well for all three cue types. And other subjects, such as subject 46 performed near chance in both the no-cue and off-frequency cue condition. It is clear that subject 46 simply could not detect the probe tone unless the cue was at the same frequency as the probe.

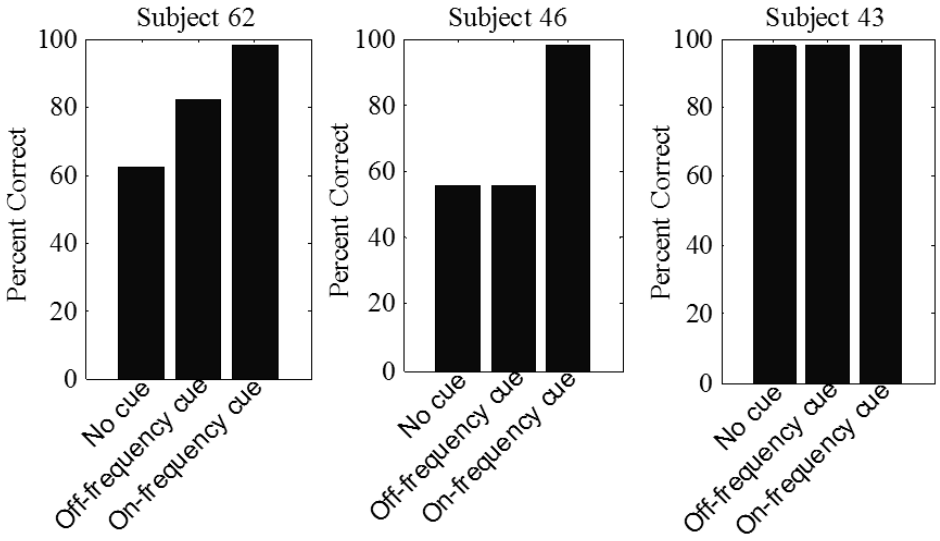

Figure 49: Results demonstrating how behavioral performance varied from subject-to-subject when using different cues to indicate presence of a target probe in noise. Subject 62 demonstrated the behavior we hypothesized – performance was highest in the on-frequency cue condition and lowest in the no-cue condition. However, Subject 43 performed equally well under all three cue conditions. Subject 46 performed only above chance when the cue was on-frequency.

3.3.2. Results of find detection threshold test

We did not find a statistically significant difference between MOC inhibition amplitudes measured during high vs. low signal-to-noise ratios during the Find Detection Threshold Test. As is shown below in Figure 50, there was no consistent and significant difference between the condition when the signal level was high and detection of the probe tone was relatively easy compared with when the signal level dropped, and detection of the probe became more difficult.

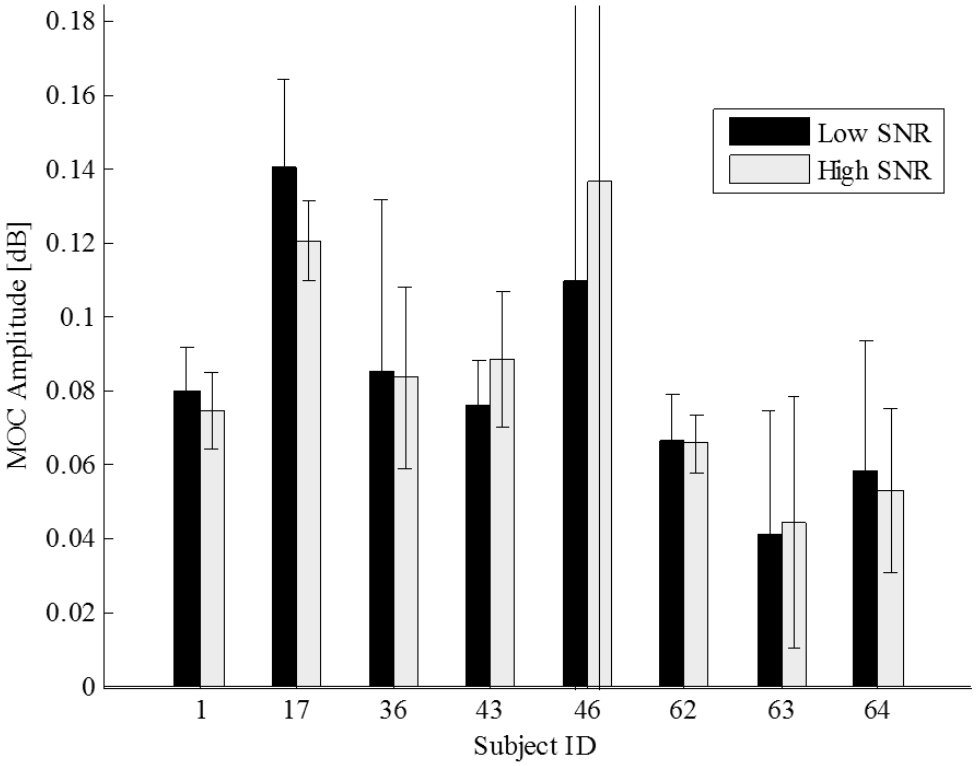

Figure 50: MOC inhibition amplitudes for each of the subjects for low SNR and high SNR. The lowest SNR was within 10 dB of their threshold at which they were achieving 90% accuracy. There was no difference observed between high vs. low SNR.

In Figure 50 above, the criteria for low SNR was based on the probe level being within 10 dB of the minimum probe level presented to the subject during the Find Threshold experiment. A high SNR was defined as when the probe level was presented at a level 10 dB greater than the minimum probe level presented to a subject during the Find Threshold experiment.

3.3.3. Results of MOC attention correlation test

Results from the Attention Correlation Test are shown in Figure 51 below. MOC amplitude is plotted across the three different cueing methodologies (no cue, off-frequency cue, and on-frequency cue) and also the difference between active and passive listening is shown. Error bars show the variability across all subjects. Despite this large variability, there is a consistent effect showing that the MOC amplitude is larger during the active listening task.

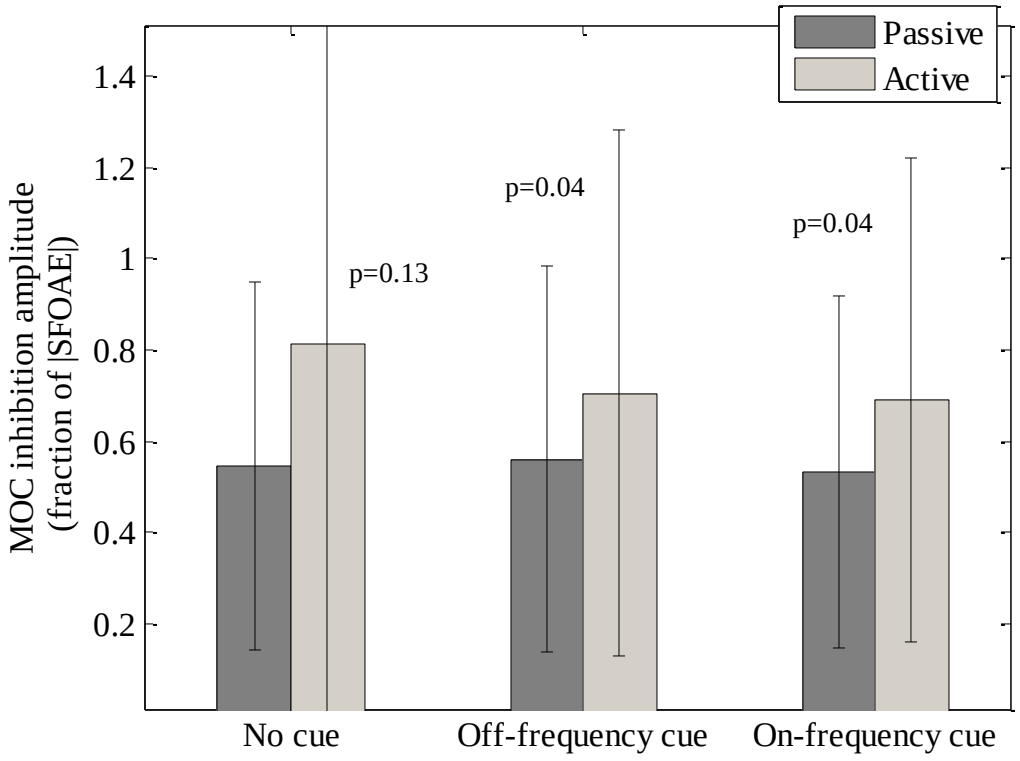

Figure 51: Averaged group results across all subjects for passive vs. active listening, and across all three cueing conditions. A paired single-tail t-test showed that the active condition MOC amplitude was larger than the passive condition MOC amplitude for the level of significance shown above. Error bars show variability in MOC amplitude across subjects. MOC amplitude is shown as the amount of total MOC inhibition of total pressure level measured in the ear during MOC activation vs. the baseline condition during which the MOC was not activated by noise.

While there did not appear to be an effect of cue on MOC inhibition amplitude, Figure 51 above shows how there was a consistent increase in the MOC amplitude during active listening. When using a single-tail paired t-test to evaluate the difference in the means, a statistically significant increase in MOC inhibition amplitude was observed in the active listening condition for both the on-frequency and the off-frequency cueing paradigms. Results from individual subjects when plotting MOC amplitude in the active vs. passive listening conditions are shown in Figure 52 below.

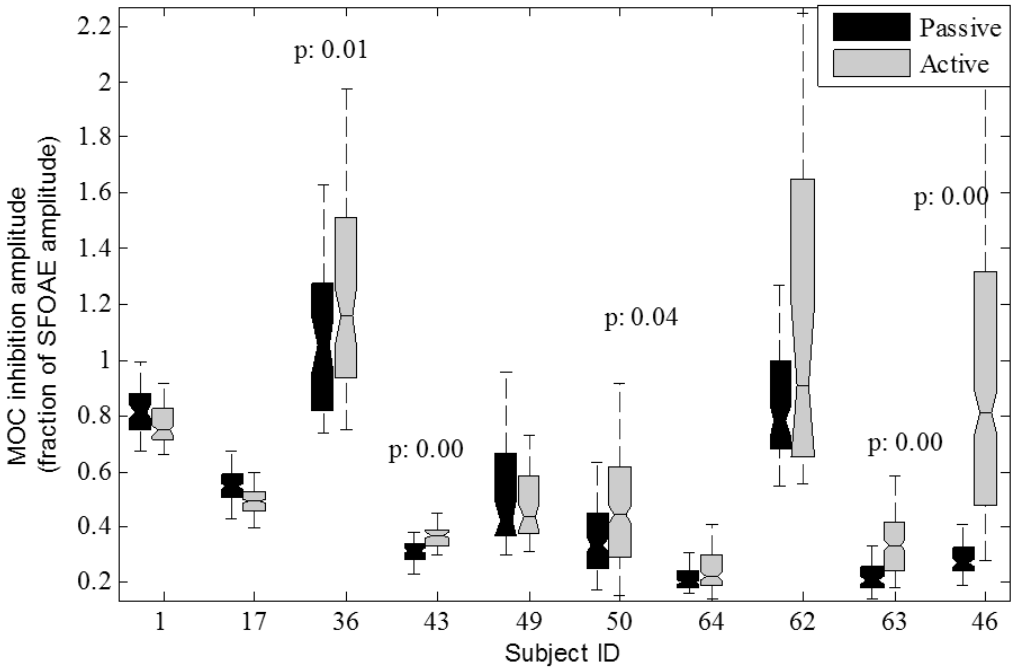

Figure 52: MOC amplitude for 10 subjects for active (red) vs. passive (blue) listening. Notice that the median MOC amplitude tends to either increase or remain the same.

The results shown above demonstrate a small but statistically significant effect of active vs. passive listening. To achieve more confidence in the significance of the effect, it is necessary to use a predictive model and use leave-one-out cross validation to determine the ability of MOC inhibition amplitude to predict active attention.

3.3.4. Prediction of active attention using MOC inhibition

Receiver operating characteristic (ROC) analysis was done to determine whether the MOC inhibition amplitude and other timing metrics can be used to predict active attention. A linear mixed effects model was fit to the data to determine the model parameters that estimate the amount that each MOC inhibition metric contribute to prediction of active attention. Subject-specific random effect variability in MOC inhibition amplitude and timing metrics were accounted for using mixed effects. The prediction model is shown below in Equation 11.

Equation 11 $$A_p(\vec{P}) = A_f + \vec{A}_r \vec{s} + \vec{P}\vec{M}$$

In this model, $A_p(\ )$ is the model estimate of active attention for a given vector of predictors (in this the predictor is the MOC inhibition amplitude). The test subjects are represented in the model by the vector ( ). is the fixed effect attention estimate when subject and predictor effect is zero. Likewise, is a vector of random effects for each subject when the predictor effect is zero. is the vector of model parameters that are found during the optimization process and they represent the amount that any predictor contributes to the prediction of active attention. The attention $A_p(\ )$ is solved for using maximum-likelihood and a simplex-based optimization algorithm. The statistical significance of each model parameter solved for is determined by calculating the p-value. Leave-one-out cross validation was used to determine prediction accuracy. In the Attention Correlation Experiment described in the previous sections, MOC inhibition amplitudes were acquired across three cue conditions (no cue, off-frequency cue, and on-frequency cue) and two attention conditions (active and passive). As is shown in Figure 51, there was no statistically significant difference in MOC inhibition amplitudes based on cue condition. Therefore, data from one cue condition (for example, on-frequency cue) was used to train the model while testing was done on other cue conditions (of-frequency cue and no-cue conditions). More specifically, in the leave-one-out cross validation, one given subject's results for one cue condition and one attention condition were left out while the model was trained on the remaining data. The left-out data set was then used as the test data set and prediction was performed on this "left-out" data set. Since each subject had data on multiple cue conditions, the random effect for a given subject could still be obtained on the training data even when one cue condition was left out.

Parameter Name	Description	Value	p-statistic
A_f	Fixed effect intercept of MOC inhibition amplitude	0.1459	0.1869
MOC inhibition amplitude (P)	Model parameter of MOC inhibition amplitude influence on active attention	0.5515	0.001

Table 8: Model parameters from fitting linear mixed effects model to active listening condition. While active listening proved to be statistically significant, the cue type condition did not, so only the model fit to active listening was fit using a mixed effects model.

Results from the ROC analysis using both a non-mixed effects model and a mixed effects model are shown below in .

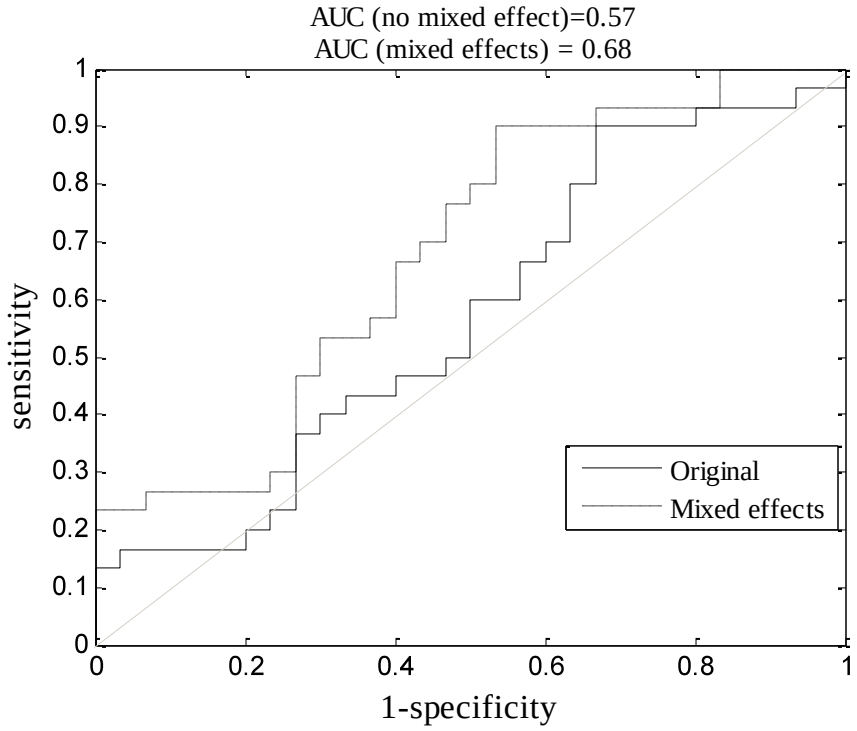

Figure 53: ROC analysis results for predicting active attention to auditory stimuli. Results are shown both before and after mixed effects are included within the model.

Adding mixed effects to the model clearly improves the prediction accuracy; AUC is 0.68 for mixed effects vs. 0.57 for non mixed effects. Further demonstration of the benefit of mixed effects is shown in Figure 54 below. The mixed effects model essentially normalizes the MOC inhibition amplitudes by removing the variability in the baseline (passive listening) condition.

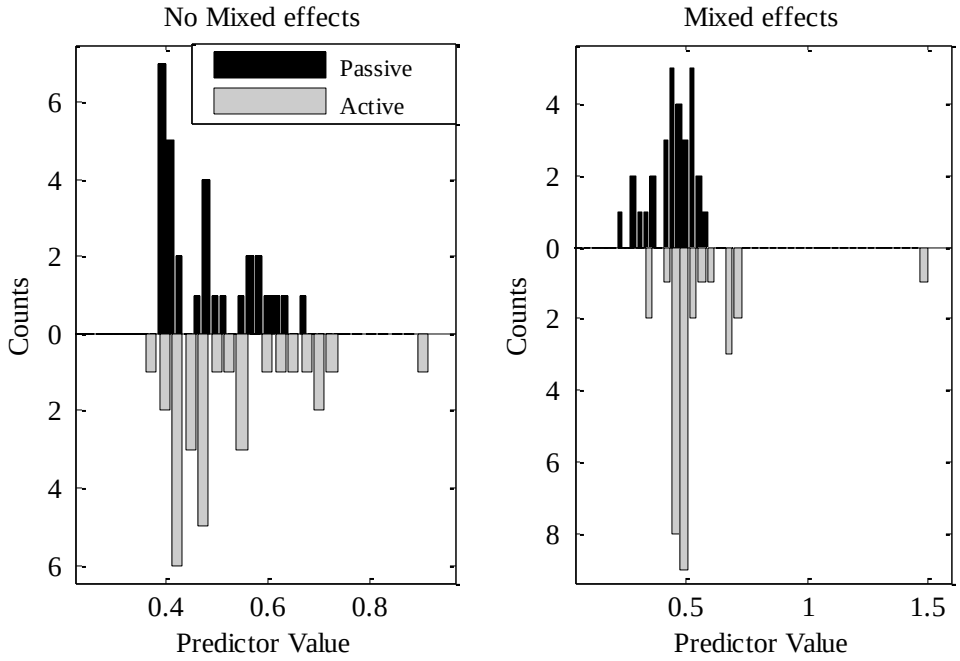

Figure 54: Histograms showing separation of MOC inhibition amplitude before and after mixed effects are included in the model. Using mixed effects demonstrates better separation of the data based on active attention.

3.3.5. Focused auditory attention model parameters within auditory model

We have presented in this chapter results that demonstrate a statistically significant effect of focused auditory attention on MOC inhibition amplitude. Results in Figure 51 show that the MOC inhibition amplitude increased on average during active attention task as compared with the passive attention task. The amount of the increase due to active attention, $\Delta V_{MOC}(a)$, is shown in Table 9 below. MOC inhibition is given as a fraction of the SF OAE amplitude that was being inhibited due to MOC activation.

Cue type	MOC inhibition (active)	MOC inhibition (passive)	Difference	p-value
No cue	0.81	0.54	0.27	0.13
Off-frequency	0.70	0.55	0.15	0.04
On-frequency	0.69	0.53	0.16	0.04
Mean	0.73	0.54	0.19	

Table 9: Change in MOC inhibition amplitude as measured by the fraction of the SF OAE inhibited during active and passive listening tasks. This is the value of $\Delta V_{MOC}(a)$ within the auditory model.

The size of the active listening effect shown in the table above is given in terms of Pa/Pa since this is a unitless effect size given in terms of the fraction of the SF OAE amplitude that is suppressed by MOC inhibition. During the attention correlation experiment, the SF OAEs were not measured. Rather, they were measured prior to the attention experiment during the screening test. The MOC inhibition amplitude is the drop in total pressure in the ear canal caused by MOC elicitation, and then scaled by the SF OAE amplitude measured in the screening test.

This change in the MOC inhibition amplitude due to attention can be represented within the Ferry & Meddis auditory model (2007) as shown below in Figure 55 .

DRNL filter bank with extensions including physiologic effects on MOC and cochlear gain

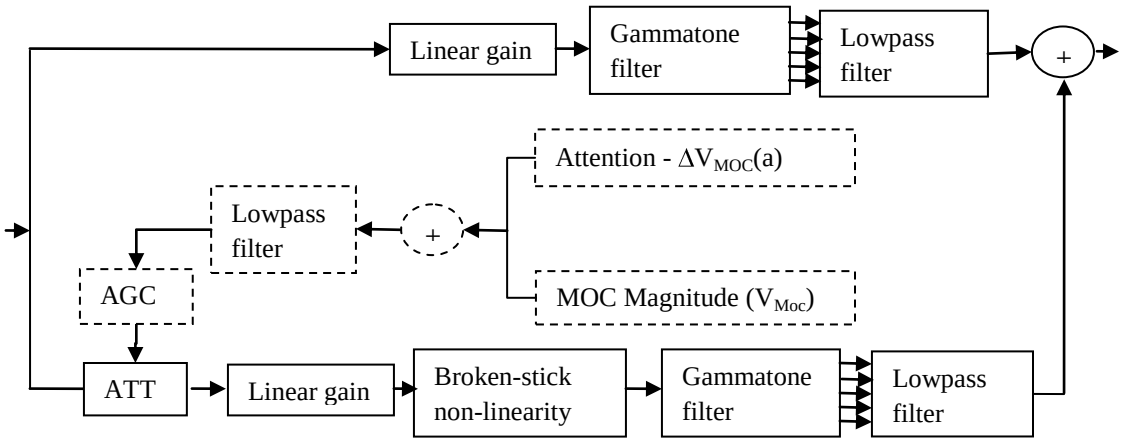

Figure 55: Ferry & Meddis model (2007) extended to show influence of focused auditory attention on the MOC inhibition amplitude.

This model was also shown in chapter 1 (Figure 13) whereby glucose was included along with attention as a factor influencing the MOC. Glucose levels of the subjects were measured at the beginning and end of this experiment to ensure that their blood glucose was below 160 mg/dL – and therefore lower than the level that was considered "hyperglycemic". Therefore, glucose levels were assumed to be constant during the attention experiment and we were able to consider attention effects on the MOC independent of glucose variability.

3.4. Discussion

Several new findings have been presented in this chapter relating to how the efferent MOC functions in different auditory attention related tasks. First, we explored whether the MOC activation changes during active listening tasks as compared with passive listening tasks. A significant increase in MOC inhibition amplitude was observed during the active listening task. Second, we explored whether three different cue conditions, previously theorized to be influential in activation of the MOC, directly influence the observed MOC inhibition amplitude. While we found that there was no effect of cue on MOC inhibition amplitude in this experiment, we discuss below a possible reason for why nothing was observed and also outline a new experimental paradigm that could be used to further explore this topic. Also in this chapter, we investigated whether signal level affected MOC inhibition amplitude by monitoring MOC inhibition during a psychophysical experiment in which probe level was adaptively reduced. We did not find a correlation of signal level with MOC inhibition amplitude. Finally, at the end of this section, we present a brief discussion regarding how attention effects should be considered relative to results presented from chapter 2 in which MOC inhibition changes were observed during hyperglycemia.

3.4.1. Influence of focused attention on MOC inhibition

We found that when an SF OAE is recorded in the ipsilateral ear while the subject attends to a tone detection task in the contralateral ear, MOC inhibition of the SF OAE increases

compared with passive listening. The increase in MOC inhibition amplitude as a result of active listening suggests the auditory cortex is being used to modulate and control the MOC to potentially improve performance in a probe-in-noise detection task. The control of the MOC via auditory pathways is something that we expected based on prior anatomical studies (Mulders & Robertson, 2002; Spangler & Warr, 1991), cochlear nucleus measurements during attention tasks (Hernandez-Peon, 1966; Hernandez-Peon, Scherrer, & Jouvet, 1956), various electrical stimulation experiments whereby stimulation of the inferior colliculus studies reduced compound action potentials in the auditory nerve (Mulders & Robertson, 2002) and finally cortical stimulation studies in which humans' auditory cortex was stimulated during surgery leading to reduced OAE amplitudes (Perrot, et al., 2006).

A natural hypothesis for explaining why the MOC inhibition would increase during active listening should be based upon whether an increase is beneficial in improving communication (Guinan Jr. J. J., 2010). In our attention experiments, the SF OAE was evoked in the ipsilateral ear, while the active listening task involved detection of tones in noise in the contralateral ear. In this test scenario, the tone that is evoking the SF OAE in the ipsilateral ear is essentially irrelevant to the test subject's task of identifying the tone. And this acoustical signal is likely regarded as distracting to the subject as they perform the active task of detecting the probe signal in the contralateral ear. Therefore, we would expect that if the amplitude of the distracting tone could be reduced, it might help the subject to perform better on the listening task. This would therefore explain why the subjects exhibited a larger MOC inhibition of the SF OAE tone in the ipsilateral ear while they were actively trying to detect probe tones in the contralateral ear. And these results are in agreement with Maison et al. (2001) who showed that MOC inhibition of OAEs in the ipsilateral ear increased when subjects were trying to detect probe tones in the contralateral ear when the probe tones were at the same frequency as the tones used to evoke the OAE. Maison et al. also found that if the frequency of the probe tone was different than the OAE

tone, then the ipsilateral inhibition amplitude was smaller. This again makes sense when considering the natural hypothesis that the MOC will be larger when it is beneficial to be larger. When the target and the OAE probe are identical, the OAE probe tone is likely more of a distracter, making it more difficult for the subject to identify the tone in the contralateral ear. And it is more beneficial to the subject's performance on the listening task that the amplitude of the ipsilateral tone is reduced by larger activation of the MOC. When the OAE probe tone is spectrally further away from the target probe, perhaps it is less of a distracter and therefore, performance enhancement is not dependent on suppression.

Results that we presented indicating an increase in MOC inhibition during active listening tasks could be interpreted as conflicting with those of de Boer and Thornton (2007). Contrary to our results, De Boer and Thornton showed that MOC inhibition decreased during the active listening task. One explanation for the difference could be the experimental paradigm. In de Boer and Thornton (2007), subjects were asked to focus their attention on tones in the ipsilateral ear, while the MOC was evoked in the contralateral ear with noise. De Boer and Thornton found that during the active listening condition when subjects were asked to focus their attention on tones in the ipsilateral ear, that suppression effects were smaller during the active task as compared with the passive task. This fits with the natural hypothesis since the subject is attempting to identify tones in the ipsilateral ear, it would not make sense to inhibit the cochlear response to these tones. Therefore, inhibition of the cochlear amplifier during the active process of searching would not make sense, and de Boer and Thornton found this to be the case – less inhibition during active tasks. If subjects in de Boer and Thornton's experiment had been asked instead to attend to tones in the contralateral ear, we might then expect that the MOC inhibition in the ipsilateral ear would be higher during the active condition. Harkrider and Bowers (2009) tested this hypothesis (having their subjects attend to the ipsilateral ear first, and then the contralateral ear) and found that there was no difference in ipsilateral vs. contralateral listening

conditions. Furthermore, like de Boer and Thornton, they also found that the MOC declined during the active listening condition. In their experiment, subjects were initially given no instructions other than to sit quietly while click-evoked OAEs were recorded both in quiet and with a contralateral MOC elicitor; this was the passive listening condition. Next, subjects were instructed to attend to the ipsilateral click stimuli that were being used to evoke the OAEs, and then finally, subjects were asked to attend to the contralateral noise stimuli. Harkrider and Bowers found that the MOC inhibition was significantly smaller (0.5 dB) during the active condition.

So how can two competing results be reconciled? In one set of work, there is a claim that MOC inhibition increases during attention tasks (Ferber-Viart, Duclaux, Collet, & Guyonnard, 1995; Maison, Christophe, & Collet, 2001) . The work presented in this dissertation is in agreement with these results. But in another set of work, results show exactly the opposite; that MOC inhibition decreases during attention tasks (de Boer & Thornton, 2007; Harkrider & Bowers, 2009). The work by Ferber-Viart et al. can be disregarded within this discussion because the attention task that they studied was a visual attention task as opposed to the other studies that all involved auditory tasks. Furthermore, the level of contralateral stimuli used by Ferber-Viart to evoke the MOC was 95 dB SPL, which is certainly large enough to evoke the MEMR in all of their test subjects. And it is likely the MEMR, which likely dominated their results, not the MOC. Regarding the other four studies, the primary differences between the de Boer / Harkrider studies and the Maison et al. / Jacobs studies, is that the studies by de Boer & Thornton and Harkrider & Bowers involved using click-evoked OAEs; and attention was being paid to either clicks or broadband noise in the active attention tasks. Contrary to that, the Maison et al. experiment as well as the results presented in this chapter involved tone-evoked OAEs and the active attention task required the subjects to pay attention to tones. The fact that tone-evoked vs. click-evoked OAEs were used in Maison et al.'s work is significant, especially considering they

only observed an increase in MOC inhibition amplitude when the tones evoking the OAE were identical in frequency to the tone they were detecting. It could be that the activation of the MOC is most useful for helping people discriminate tones or tone-like acoustical information such as speech as has been shown by Kumar and Vanaja (2004). But when broadband stimuli are being detected such as noise or clicks, it is not helpful for the MOC to be activated so there is less activation during these types of stimuli. Kumar and Vanaja (2004) showed that speech recognition in subjects improved in the presence of noise at certain signal-to-noise ratios. And other work by de Boer and Thornton (2008) showed that efferent activity can actually predict whether certain subjects will demonstrate improvement in speech-in-noise discrimination tasks. The type of OAEs being evoked could also be significant. A click-evoked OAE represents a summed response along the entire length of the basilar membrane, whereas a tone-evoked OAE is a response from a precise location on the basilar membrane. Therefore, inhibition of the click-evoked OAE through MOC activation is overall a grosser measurement, lacking in spatial selectivity along the basilar membrane. This spatial and spectral specificity may end up being a critical factor influencing how the auditory cortex influences activation of the MOC.

3.4.2. Affect of cue conditions on accuracy and on MOC inhibition

Behavioral results from the psychophysical testing generally followed what we would have expected to observe. As shown in Figure 48, on average, the subjects performed better when the cue frequency was the same as the probe tone that they were attempting to identify. And they performed the worst under the condition where there was no cue given at all. However, not all of the subjects followed this pattern as is shown for several specific subjects in Figure 49. But overall, subjects generally followed behavioral trends that we expected. And therefore, it was somewhat surprising that there was no effect of the cue condition on the MOC inhibition amplitude. However, recall from section 3.1.3 that we described two possible explanations for why people perform better in on-frequency cue vs. off-frequency cue conditions. The first

explanation for cue-based performance enhancement is that the presentation of the cue causes increased MOC inhibition outside of the cue's critical bandwidth. This clearly did not happen in the experiment described in this chapter, since if it did, we would have observed a larger MOC inhibition amplitude during the presentation of the cue vs. the no-cue condition. The second explanation for improved performance is that the cue causes a release from MOC inhibition within the cue's critical band thereby enhancing the cochlear response within the critical band of the cue. We did not test this hypothesis specifically in the experiment described in this chapter because the tone used to evoke the SF OAE was spectrally distant from both the cue and the probe tone. This was done so as not to interfere with the psychophysical probe detection task. However, if the SF OAE elicitor tone was within the critical band of the probe, it would help to answer the question of whether such a release from inhibition is being generated by the cue. It would also be interesting to elicit the MOC in the same ear as the detection task to better understand whether the effect is dependent upon which ear the detection task is being done in relative to the ear where the SF OAE is being elicited. A forward masking paradigm could be used with a post-elicitor window as was done in the glucose experiments described in chapter 2.

3.4.3. Effect of signal-to-noise ratio on MOC inhibition

The purpose of the Find Detection Threshold Experiment was primarily to find the signal level at which the test subjects were barely achieving 100% accuracy during the detection task. This detection level was what we used to present probe tones in the Attention Correlation Experiment. However, we were also interested in whether the MOC inhibition amplitude changed based on changes in the signal-to-noise ratio. We hypothesized that perhaps the MOC would increase as the SNR declined, because the MOC inhibition of noise could potentially improve performance of tone detection. We did not find a correlation of MOC inhibition amplitude with SNR level. Two of the subjects showed a slight increase in MOC inhibition amplitude as the SNR declined during the Find Detection Threshold Experiment. But the effect

was not large, and none of the other subjects demonstrated this. These results suggest that while the MOC may get activated by the auditory cortex to improve tone detection in noise, it is not sensitive enough to change based on changes in signal level.

3.5. Attention influence on MOC inhibition during glucose testing

Results have been presented in this chapter showing that for a majority of subjects tested, the MOC tends to be larger when the subjects are actively attending to auditory stimuli. The effect was statistically significant in 5 out of the 10 subjects tested. In chapter 5 (section 5.2.1), we compare the size of MOC inhibition during active attention with the size of MOC inhibition during hyperglycemia to compare the relative changes. In Table 15 in chapter 5, a comparison is made between glucose vs. attention influences on MOC inhibition amplitude, and the effect size is shown to be comparable. Results from this chapter and the data shown in Table 15 indicate that controlling a patient's attention may be important if the MOC is ever to be used within a clinical setting. The diabetes subjects tested in chapter 2 had their attention controlled during the glucose tolerance test; they were required to watch silent movies during the testing. Subjects who did not consume sugar during the glucose tolerance test, but only watched silent movies, did not demonstrate a statistically significant change in MOC inhibition during the course of watching the movie (as shown in Figure 32-Figure 34). These control runs suggest that changes in MOC inhibition amplitude that occurred during the diabetes subjects' glucose tolerance tests were caused by elevated blood glucose and not by changes in the subjects' attention to the audio or visual stimuli. For the nondiabetic subjects tested, their attention was not as well controlled, and so it is difficult to assess whether their attention changed during the glucose tolerance testing. For all future testing of the MOC, results from this dissertation indicate the importance of controlling the subject's attention during test sessions.

Chapter 4

4. Effects of aging on SF OAE amplitude and latency

In chapter 2, we demonstrated that there is a significant effect of glucose on the MOC that is likely being driven by ATP-sensitive ion channels, and in chapter 3 we demonstrated that the MOC can be cortically controlled by instructing listeners to focus their attention on a specific listening task. A final topic presented in this dissertation is focused on the influence that aging has on SF OAEs. MOC inhibition data was not available within this test protocol due to time constraints, so it was not examined relative to aging. In this experiment, many of the same signal extraction and statistical modeling techniques described earlier were used to evaluate whether age-related hearing changes influence OAE amplitude and latency.

The impact of aging on the auditory system is not fully understood. Presbycusis, or age-related hearing loss, can be hypothesized to be related to anatomical changes within the cochlea as is discussed by Stover and Norton (1993). Changes in the cochlea due to aging can potentially lead to sensory changes, metabolic changes, and mechanical changes to the basilar membrane and middle ear. Sensory changes as a result of presbycusis can be a result of a decrease in the number of outer hair cells and also a change in the outer hair cells that remain intact as a result of aging (Anniko, 1988). Metabolic changes due to presbycusis involve metabolic factors including atrophy and degeneration of the stria vascularis. Such atrophy of the stria vascularis can lead to a decrease in the endocochlear potential (EP), which has been shown to lead to smaller OAE amplitudes (Mills, Norton, & Rubel, 1993). Finally, mechanical changes due to presbycusis have been proposed such that the basilar membrane stiffness may change with aging.

In this chapter, we investigate whether aging impacts the auditory system independent of hearing by examining SF OAE amplitude and latency measures in both younger and older adults with normal hearing thresholds. We have found that aging has a consistent and significant impact

on SF OAE amplitude and a small, though not statistically significant effect on SF OAE latency. A statistical model is presented that describes suppressor, probe and aging impacts on the SF OAE amplitude and latency measures.

4.1. Background

4.1.1. Hearing changes with aging

There has been a lot of work demonstrating that hearing functionality declines as people age. Depending on the frequency of the auditory signal being tested, different groups have reported a rate of increase of pure-tone thresholds in older adults of between 5.5 to 9 dB/decade (Davis, Ostri, & Parving, 1990; Gates, Cooper, Kannel, & Miller, 1990; Ostri & Parving, 1991; Divenyi, Stark, & Haupt, 2005). In addition to using pure-tone threshold as a measure of hearing change with age, some groups have demonstrated a decline in speech perception in both noisy and quiet environments in older adults (Blumenfield, Befgman, & Millner, 1969). There is data showing that speech understanding in noise gets progressively worse with aging (Gelfand, Piper, & Silman, 1986; Dubno, Lee, Matthews, & Millis, 1997), but some labs have provided conflicting evidence that shows that performance on speech recognition tasks in quiet do not change significantly with aging (Tun, 1998; Gelfand, Piper, & Silman, 1985).

It is difficult to distinguish age-related hearing changes and hearing loss due to other factors such as exposure to noise because as people get older, there is more opportunity for exposure to noise and therefore hearing loss can occur through this exposure. This has made it difficult to study aging impacts on the auditory system that are independent of hearing impairment. However, studies indicate that there may be changes to the auditory system that occur due to aging that are independent of hearing loss. Gelfand et al. (1986) demonstrated that when subjects with normal pure-tone thresholds were evaluated in consonant recognition tests in noise, performance deteriorated with aging. Dubno et al. (1997) did a similar test while

evaluating speech recognition accuracy in noise for old and young subjects and found that performance decreased significantly with aging in men only, but not in women.

4.1.2. OAE amplitude changes with aging and hearing impairment

The effect of aging on the auditory system can be further explored using physiologic measures by examining OAE amplitudes in older and younger subjects. OAEs provide information about outer hair cell functionality in the cochlea (Kemp, 1978) and the amplitude of distortion product OAEs has been shown to decrease and progressively disappear as a result of hearing damage and resulting elevated hearing thresholds (Martin, Ohlms, Franklin, Harris, & Lonsbury-Martin, 1990; Gorga, et al., 1993; Kimberly, Hernadi, Lee, & Brown, 1994). While groups have examined aging effects on OAE amplitude, results have been mixed and oftentimes contradictory (Strouse, Ochs, & Hall, 1996; Dorn, Piskorski, Keefe, & Neely, 1998; Chida, 1998; Stover & Norton, 1993; Bonfils, Bertrand, & Uziel, 1988; Collet, Moulin, & Morgon, 1990; Hoth, Gudmundsdottir, & Plinkert, 2010).

Bonfils et al. (1988) and Collet et al. (1990) both found that while evoked emissions were present in 100% of subjects under the age of 60, the incidence fell to 35% after age 60. Hearing impairment was not adequately controlled for in either of these studies and it was impossible to know whether the change in evoked emissions was due to aging or hearing impairment. Strouse et al. (1996) found that when peripheral hearing loss was adequately controlled for, there was no direct effect of aging on DP OAE measure. However, Dorn et al. (1998) noted that there was significant variability of Strouse's inter-subject DP OAE threshold for the younger subjects, and that this variability may have obscured the aging effect. In Dorn et al.'s study (1998), they used multivariable regression analysis and found that age, frequency, and threshold all showed a correlation with reduced OAE amplitudes. They observed a significant relationship between age and frequency for both older subjects and younger subjects, but no significant interaction of age and threshold. Hoth et al. (2010) more recently looked at two groups of subjects; one group had

no age-related hearing loss, and the second group that did have age-related hearing loss. The OAE amplitude reduction with aging was consistent across all frequencies and more pronounced for the group that did show presbycusis than for the group that did not, thereby indicating that it is the hearing loss itself that is responsible for OAE amplitude reductions.

There has been less work examining SF OAE measures with aging as compared with DP OAEs. In recent years, however, more work has been done examining how SF OAEs can be used to predict hearing loss. Ellison et al. (2007) was able to predict hearing thresholds using SF OAE amplitudes and middle ear measurements with reasonable accuracy. They found that SF OAE signal-to-noise ratio was the best predictor of hearing impairment, but SF OAE amplitude was also a good predictor. Ellison et al. did not investigate aging effects on their prediction methods.

Because of the prior experiments demonstrating that hearing becomes impaired with aging, we hypothesized that SF OAE amplitudes will be reduced in older subjects.

4.1.3. OAE latency changes with aging and hearing impairment

As with OAE amplitudes, there have been several studies that have investigated the effect of hearing loss on OAE latency. There have not been any consistent results demonstrating an effect of long-term hearing changes and OAE latency (Prijs & Schoonhoven, 1997; Ramotowski & Kimberley, 1998; Hoth & Weber, 2001). Ramotowski and Kimberley (1998) did not find statistical significance of mild hearing loss with DP OAE latency; however they did find a slight increase in traveling wave delay with aging that was statistically significant. Hoth and Weber (2001) also found no correlation of TE OAE or DP OAE latencies with hearing impairment but did find that ABR latencies increased by a small but significant amount in hearing impaired subjects. They noted a large variability in their latency measurements that could have potentially hidden any underlying effects. Enghal and Kemp (1996) looked at group delays of DP OAEs and found that they decreased in subjects exposed to a temporary noise-induced hearing loss.

SF OAE latency measures may provide a noninvasive estimate of cochlear tuning associated with outer hair cell function as well. SF OAE latency is hypothesized to be a measure of the round-trip travel time between the ear canal and tonotopic place region on the basilar membrane (Kemp, 1978) and this latency is thought to be inversely proportional to the local value of the auditory filter bandwidth, Q_{erb} (Zweig & Shera, 1995). In support of this prediction, SF OAE group delay based estimates of Q_{erb} for 40 dB SPL tones vary with stimulus frequency and predict psychophysical Q_{erb} in normal ears (Shera, Guinan, & Oxenham, 2002). Furthermore, SF OAE onset latency based estimates of Q_{erb} vary with stimulus level and hearing status. This study was partly motivated by evidence that outer hair cell function assessed using psychophysical two-tone suppression is reduced in older adults with normal hearing (Dubno & Ahlstrom, 2001), suggesting that aging may impact measures of cochlear tuning independent of hearing loss and/or that subclinical changes in hearing may be associated with abnormal tuning.

4.1.4. Effect of stimulus level on SF OAE amplitude and latency

When investigating the effect of aging and hearing impairment on SF OAE measures, it is important to also model the effect of probe and suppressor stimulus level on the SF OAE. Prior work by Konrad-Martin and Keefe (2005) demonstrated that SF OAE amplitude tends to increase with probe level. They also showed that SF OAE latency tended to decrease with probe level. This work was consistent with other work that also showed that latencies decrease with stimulus level (Whitehead, Stagner, Martin, & Lonsbury-Maertin, 1996; Konrad-Martin & Keefe, 2003). Reports from other labs studying DP OAEs have also shown that group delay decreases with increasing stimulus level (Bowman, Brown, Eggermont, & Kimberley, 1997; Wable, Collet, & Chery-Croze, 1996; Kimberley, Brown, & Eggermont, 1993; Bowman, Eggermont, Brown, & Kimberley, 1998) . This study extends the prior work by modeling probe and suppressor level effects on OAEs while also looking at how this relationship might change with aging and hearing impairment.

4.2. Methods

In this study, we compared SF OAE amplitudes and latencies in 32 younger (<50 years) and 21 older subjects (>50 years) with hearing ranging from normal to mild hearing impairment. The age distribution of the subjects tested is shown below in Figure 56.

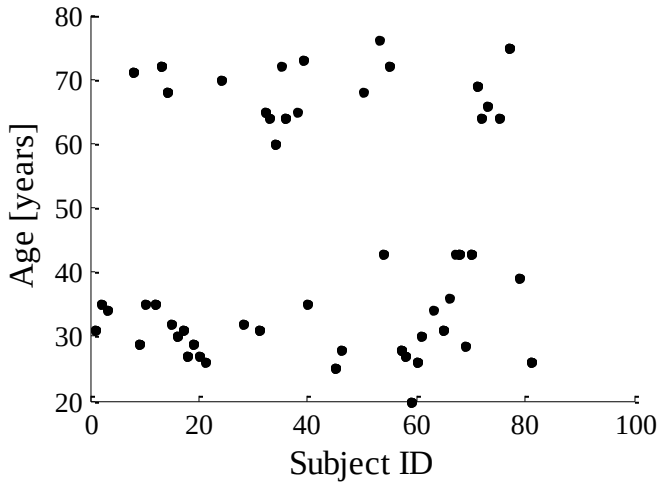

Figure 56: Age distribution of subjects tested.

SF OAEs were elicited using a double-evoked method (Keefe, 1998; Keefe & Ling, 1998) whereby a 110 ms suppressor tone (S1) was presented first, followed by a 100 ms probe (S2), each centered within a 1-second time window. And finally, both suppressor and probe were presented simultaneously (S12). The SF OAE residual (Pd) is estimated using Equation 1. The probe frequency in this experiment was chosen to be 3175 Hz. The frequency of the suppressor tone was selected as 0.97 times the probe frequency. Probe level varied from 40 to 65 dB SPL in 5 dB increments. Three suppressor levels were tested; 5, 10, and 20 dB greater than the probe level. Signal processing of SF OAE waveforms was identical to the methods described in section 2.2.7.

4.2.1. Linear mixed effects model of SF OAE amplitude and latency

A linear mixed model was used to fit the observed SF OAE response variables (latency and amplitude) as a function of age, probe level, and suppressor level. The model equation for predicting latency based on the model parameters with all covariates is shown in Equation 12.

Equation 12

$$L = L_0 + L_{age} * Age + L_{Supp} * Supp + L_{probe} * probe + \sum_{i=1}^{Covariates} L_{covi} * Cross\_Term_i$$

Where:
L: Latency estimated by model
Supp: Suppressor level in dB SPL
Probe: Probe level in dB SPL
Age: Age of subject in years
Cross_Term: Covariate of probe, suppressor, and age with each other

And the six model parameters are:

L_0:	Intercept latency estimate
L_{age}:	Age influence on latency estimate
L_{Supp}:	Suppressor level influence on latency estimate
L_{Probe}:	Probe level influence on latency estimate
L_{Cov}:	Covariate influence on latency estimate

Subject variability was estimated using random effects within the mixed effects model. In the mixed effects model, subject-level variability was estimated for only the intercept term (L_0) as the assumption was made that the age, probe level and suppressor level would remain fixed across all subjects. The intercept term consisted of a random effect and a fixed effect. The intercept term therefore has a different value for each subject (s) as shown below.

Equation 13 $$L_0(s) = L_0^{Fixed} + L_0^{Random}(s)$$

Random effects were not estimated for the covariate parameters.

The same was done for the SF OAE amplitude estimation model.

Equation 14 $$A = A_0 + A_{age} * Age + A_{Supp} * Supp + A_{probe} * probe + \sum_{i=1}^{Covariates} A_{covi} * Cross\_Term_i$$

Equation 15 $$A_0(s) = A_0^{Fixed} + A_0^{Random}(s)$$

Where:
A: SF OAE amplitude estimated by model
Supp: Suppressor level in dB SPL
Probe: Probe level in dB SPL
Age: Age of subject in years
Cross_Term: Covariate of probe, suppressor, and age with each other

And the six model parameters are:

A_0:	Intercept amplitude estimate
A_{age}:	Age influence on amplitude estimate
A_{Supp}:	Suppressor level influence on amplitude estimate
A_{Probe}:	Probe level influence on amplitude estimate
A_{Cov}:	Covariate influence on amplitude estimate

The model is identical to that for latency, with the only exception being that amplitude becomes the metric being predicted.

The full models shown in Equation 12 and Equation 14 were reduced using an F-test based model identification method (Abel el-sallam & Zoubir, 2003). Model reduction began with a prediction estimate done such that the log-likelihood estimate was obtained. P-values were obtained for each of the model parameters found. The model parameter with the largest p-value was identified. If this model parameter was not present as a covariate in any of the remaining predictors, then it was removed from the predictor list. If this predictor was present as a covariate, the predictor with the next-largest p-value was considered for removal until a predictor was found that was both large and was not represented as a covariate in the list of remaining predictors. A new prediction was done and the log-likelihood estimate and p-values for each model parameter were obtained. This process was repeated until all of the model parameter p-values were under 0.05 and therefore deemed to be statistically significant. This process of model identification is shown in Figure 57.

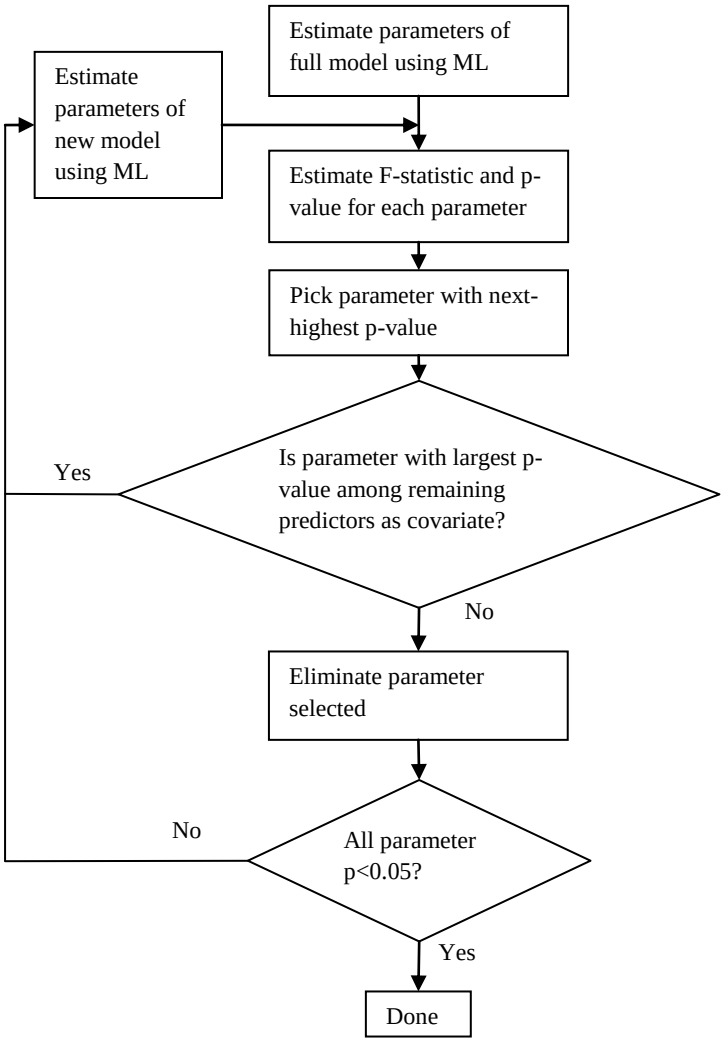

Figure 57: Model identification using F-test backward elimination method. Method adapted from Abel el-sallam & Zoubir (2003).

The reduced models, used to predict SF OAE latency and amplitude using only probe and suppressor level, are shown in Equation 16 and Equation 17. These models were used to demonstrate the effect of probe level and suppressor level across all subjects without considering age as a factor.

Equation 16 $$L = L_0 + L_{Supp} * Supp + L_{probe} * probe$$

Equation 17 $$A = A_0 + A_{Supp} * Supp + A_{probe} * probe + A_{Probe\_Supp} * probe * Supp$$

When including age as a predictor, the reduced models are given in Equation 18 and Equation 19.

Equation 18

$$L = L_0 + L_{Age} * Age + L_{Supp} * Supp + L_{probe} * probe$$

Equation 19

$$A = A_0 + A_{Age} * Age + A_{Supp} * Supp + A_{probe} * probe + A_{Probe\_Supp} * probe * Supp$$

Notice that amplitude correlated with the covariate of probe and suppressor level, but latency did not.

4.3. Results

Results are organized into the following sections. First, we show effects of stimulus level (probe and suppressor) on both SF OAE amplitude and latency will be presented. Next, mean data across all subjects are presented to demonstrate aging effects on amplitude and latency across different stimulus levels. Results of the linear mixed effects models are shown so that the effect of aging on SF OAE amplitude and latency can be quantized. And finally we show results from the ROC analysis demonstrating prediction of older age (>50 years) using SF OAE amplitude and latency.

4.3.1. Effect of stimulus level on SF OAE amplitude and latency

An ANOVA analysis showed that stimulus probe and suppressor level strongly correlated with both SF OAE amplitude and latency. The covariate of probe and suppressor level did have statistical significance with SF OAE amplitude, but the covariate term did not have significance with the SF OAE latency term. The p-values from the ANOVA are given in Table 10 below.

Predictor	p-value
Probe	<0.001
Suppressor	0.025
Probe * Suppressor	<0.000

(a) Amplitude

Predictor	p-value
Probe	<0.001
Suppressor	0.0015

(b) Latency

Table 10: ANOVA results when looking at stimulus probe and suppressor level on SF OAE amplitude (a) and latency (b).

A mixed effects model using subject ID as the random effect showed that SF OAE amplitude tends to increase with both probe and suppressor level while SF OAE latency tends to decrease with probe and suppressor level. The change in SF OAE amplitude with probe and suppressor levels is shown in Figure 58.

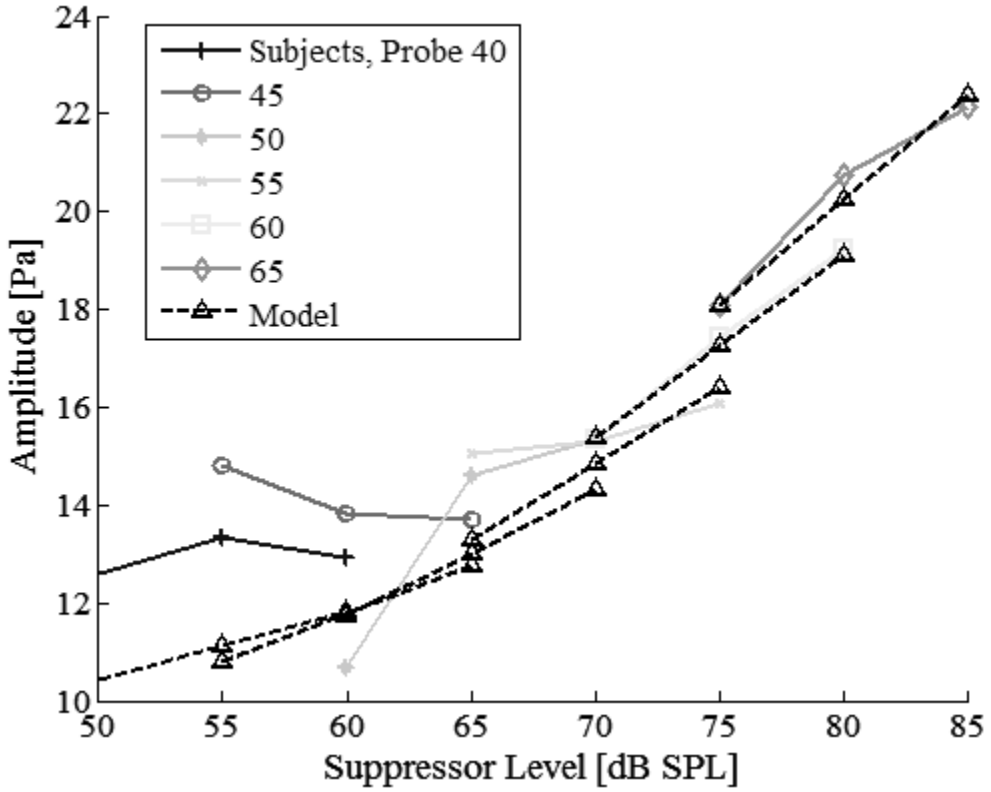

Figure 58: Mixed effects model of SF OAE amplitude using probe and suppressor level as the predictor. Model is shown in black (- -) while the actual data is solid and color-coded by probe level. This model was fit to only the young normal hearing (PTT<=25 dB HL) subjects.

Notice that the data fits the model reasonably well. SF OAE amplitude tends to increase for larger probe levels, and increase with higher suppressor levels as well. Parameters from fitting the data to Equation 14 using a linear mixed effects model as shown above in Figure 58 are given in Table 11 below.

Parameter	Value [units]	p-statistic
Intercept	31.7 [dB SPL]	<0.001
Probe level	-0.71 [dB / dB]	<0.001
Suppressor level	-0.33 [dB / dB]	0.004
Suppressor * Probe	0.01 [dB / dB]	<0.001

Table 11: SF OAE amplitude model fixed-effect parameters with probe and suppressor level as predictors.

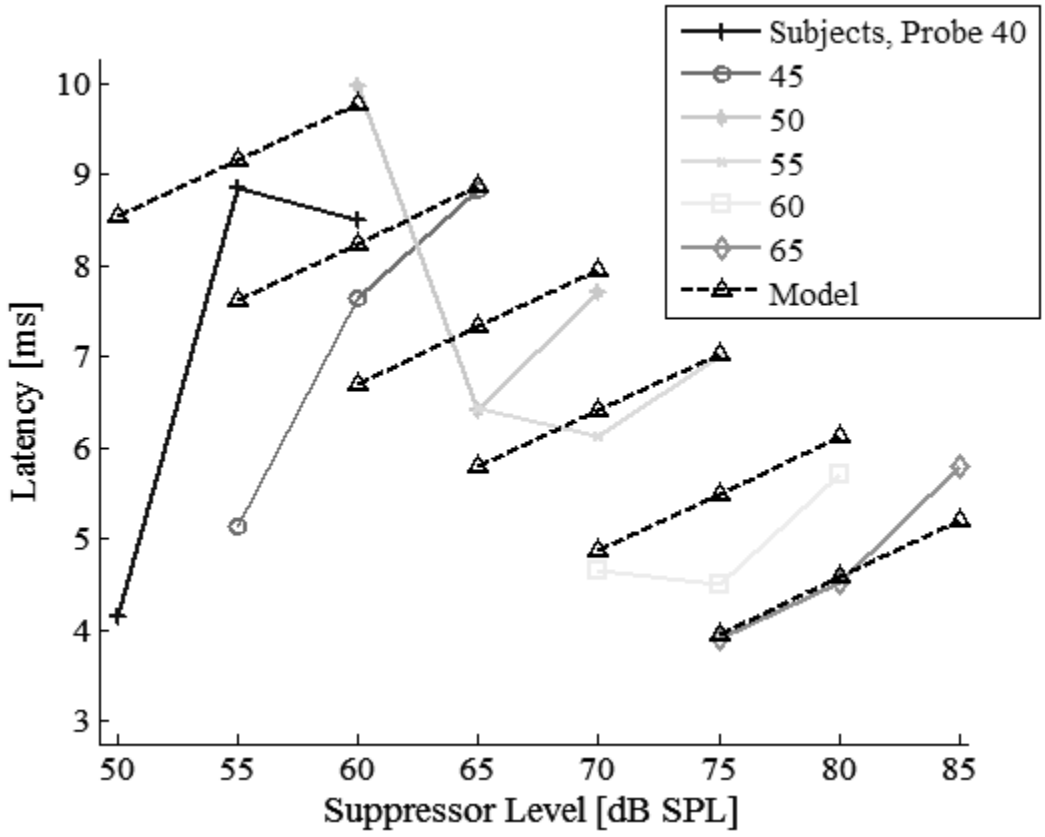

Figure 59: Mixed effects model of SF OAE latency using probe and suppressor levels as predictors. Model is shown in black (- -) while the actual data is solid color-coded for probe level. Only normal hearing subject data (PTT<25 dB HL) were used to fit the model.

The model parameters as fit to Equation 12 are given below in Table 12.

Parameter	Value [units]	p-statistic
Intercept	14.73 [ms]	<0.001
Probe level	-0.3436 [ms/dB]	<0.001
Suppressor level	0.1486 [ms/dB]	0.0011

Table 12: Model parameters for predicting SF OAE latency using stimulus probe and suppressor levels. Data fit to normal hearing subjects.

4.3.2. Influence of aging on SF OAE amplitude

Older subjects with good hearing (pure-tone thresholds under 25 dB HL) had a significantly smaller SF OAE amplitude than younger subjects. This result, illustrated in Figure 60 below, is in agreement with prior work (Dorn, Piskorski, Keefe, & Neely, 1998; Hoth & Weber, 2001; Ellison & Keefe, 2007) showing OAE amplitude reduction with increasing age.

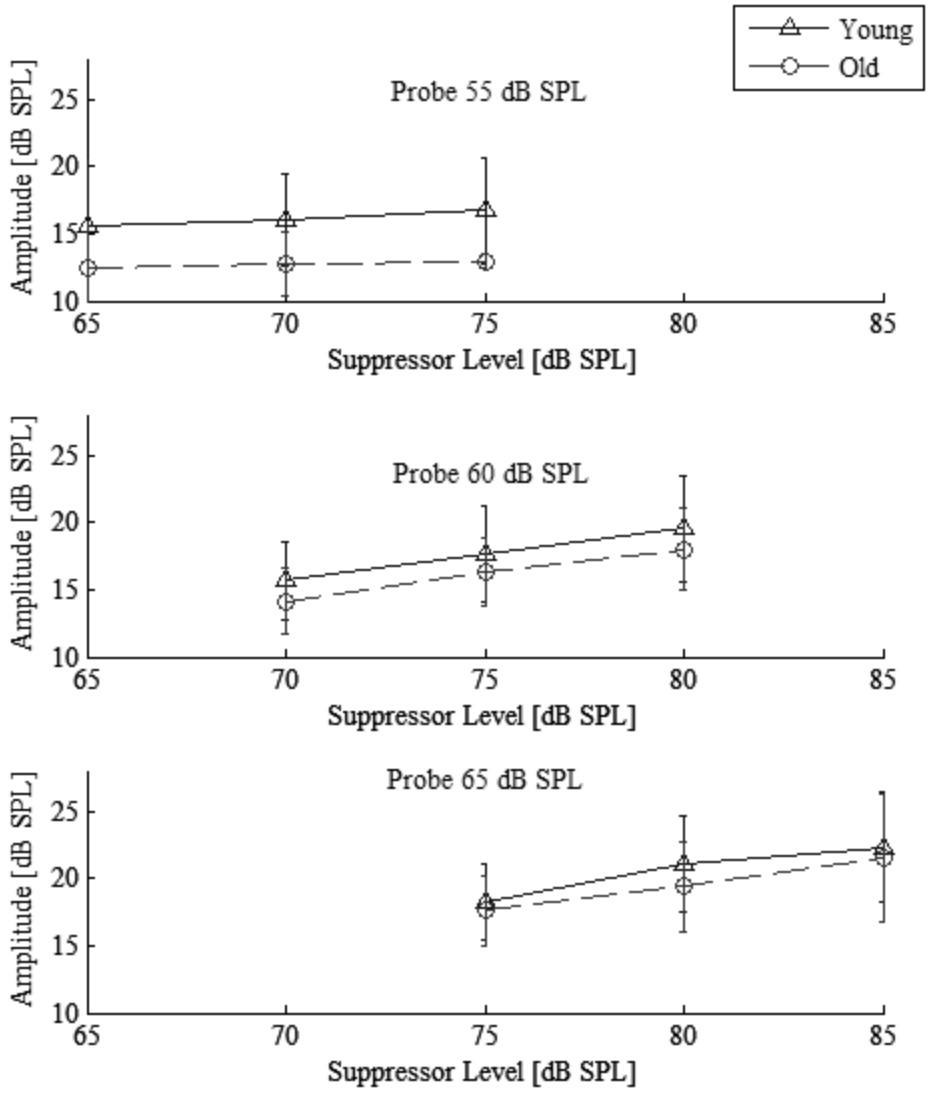

Figure 60: SF OAE amplitudes plotted for younger (age<50) and older subjects. A consistent and statistically significant drop in SF OAE amplitude is observed for the older subjects. However, the difference between old and young subjects appears to be larger at the lower probe level.

There was not sufficient data for older subjects at lower probe levels so only probe levels of 55, 60, and 65 dB SPL are shown. Fitting the linear mixed effects model in Equation 17 to the SF OAE latency data for old and young subjects with good hearing (PTT<=25 dB HL) resulted in model parameters for each of the predictors that were statistically significant. The fit of the model to the data demonstrating the aging effect is shown below in Figure 61.

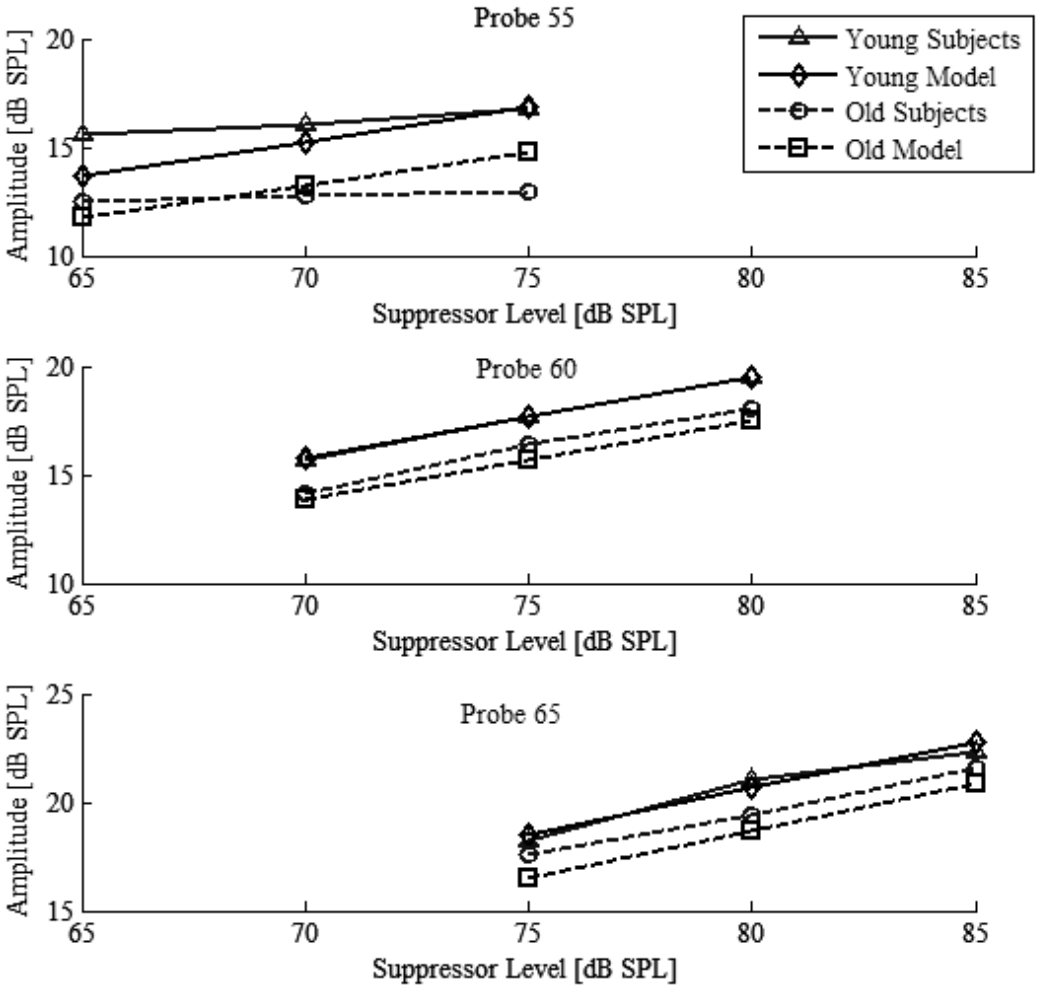

Figure 61: Aging effect on SF OAE amplitude. Linear mixed effects model is shown alongside mean data across old and young subjects. Model parameters for age, probe level, and suppressor were all statistically significant.

The parameters of the linear mixed-effects model as shown in Figure 61 are given in Table 13.

Parameter	**Value [units]**	**p-statistic**
Intercept	33.24 [dB]	<0.001
Age	-0.0519 [dB/year]	0.0377
Probe Level	-0.6973 [dB/dB]	<0.001
Suppressor Level	-0.3226 [dB/dB]	0.0218
Probe * Suppressor	0.0116 [dB/dB^2]	<0.001

Table 13: Linear mixed effects model parameters for predicting SF OAE amplitude using age, probe level, and suppressor level.

4.3.3. Influence of aging on SF OAE latency

The effect of aging on latency was not statistically significant. However, results are shown here for the sake of completeness, and also because the amount of change in latency due to aging was found to be comparable in size as compared with Ramotowski & Kimberley (1998), who measured traveling wave delay in normal and older adults.

Overall, SF OAE latency results show a general trend for SF OAE latencies to be larger for older normal hearing (PTT <25 dB HL) subjects. This trend is displayed in the mean latency plots in Figure 62.

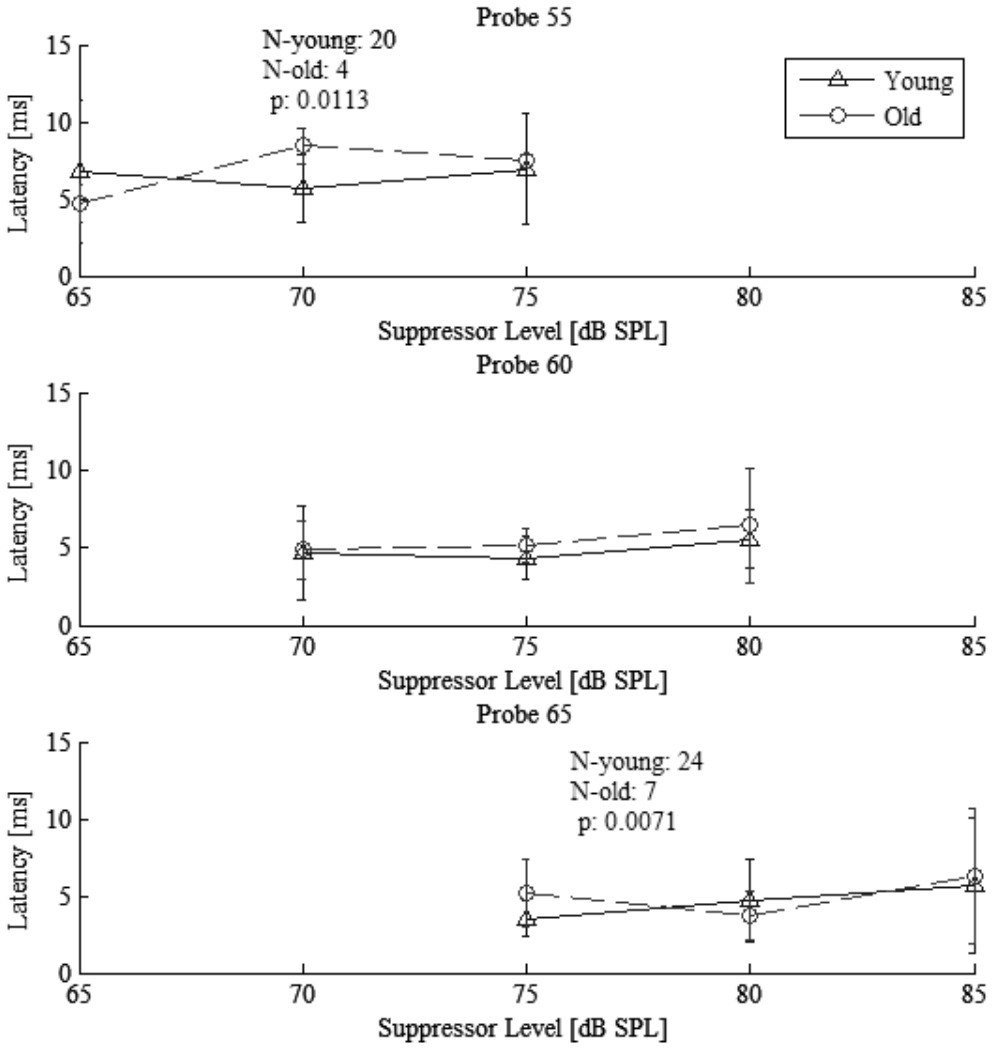

Figure 62: SF OAEs generally tended to increase in older adults across nearly all higher level probe/suppressor combinations. The increase was statistically significant in several instances.

When the linear mixed effects model in Equation 18 was fit to the latency data results are shown below in Figure 62.

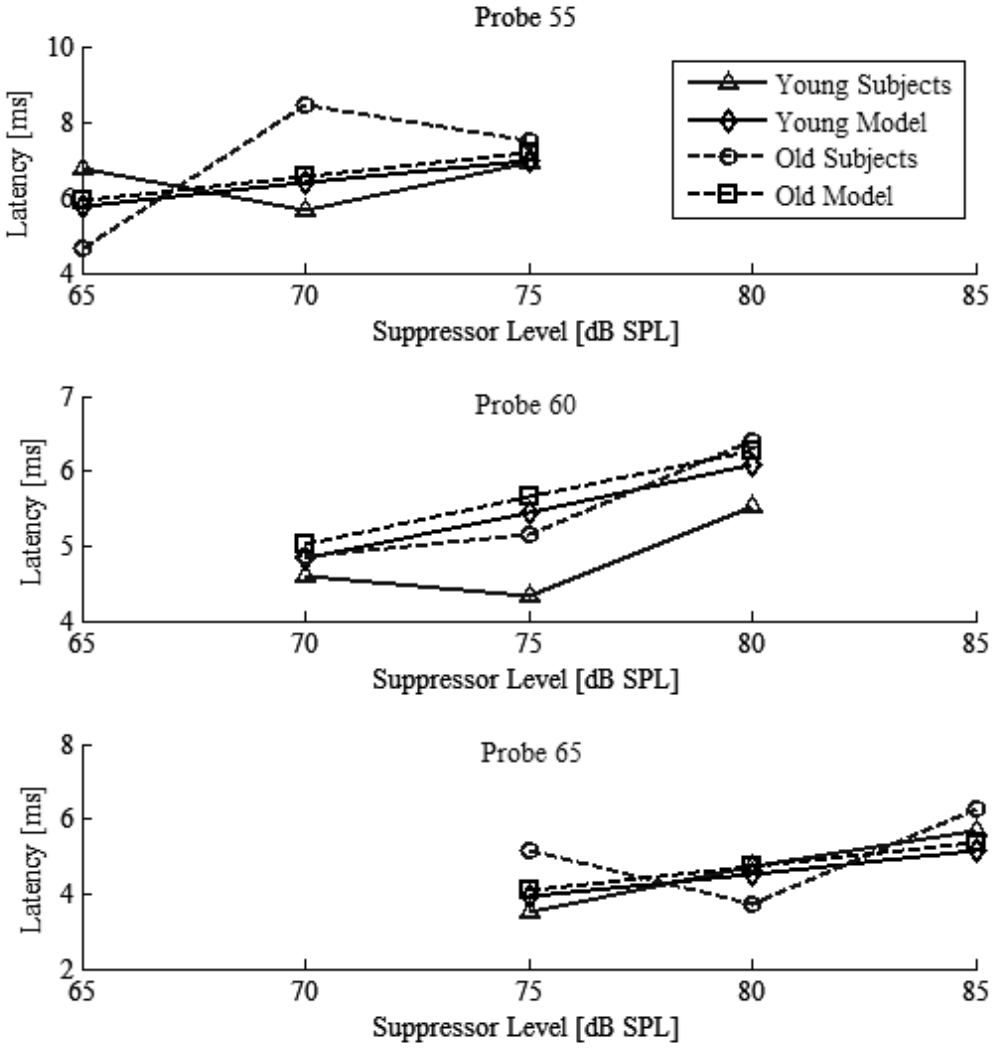

Figure 63: Effect of aging on SF OAE latency as shown by both mean data and also linear mixed effects model prediction. The aging effect (slightly longer SF OAE latency for older subjects) was found to be small and statistically not significant.

Since the influence of aging on SF OAE latency was not found to be statistically significant, we do not present the fixed effect model parameters for predicting SF OAE latency. Though not statistically significant, the SF OAE latency was found to be approximately 0.2 ms longer in the older subject group than in the younger subject group based on results from the fixed effect model. This amount of change is consistent with prior work. Ramotowsky and Kimberley

(1998) found that older subjects had slightly longer OAE latencies than younger subjects; the change was on the order of 0.2-0.3 ms.

4.3.4. ROC prediction of older age using SF OAE amplitude and latency

A clinical evaluation test utilizing receiver operating characteristic (ROC) curve analysis was done to assess whether older age (>50 years) could be accurately predicted using SF OAE amplitude and/or latency as the metric for prediction. The prediction model used was logistic regression whereby older age was predicted based on SF OAE amplitude or latency. The area-under-the-curve (AUC) was used as a model fit success criteria (Zweig & Campbell, 1993). A leave-one-out cross validation was done to determine the accuracy of the ROC and model reduction was done based on the one-standard deviation rule (Hastie, Tishirani, & Friedman, 2009) as was done when predicting hyperglycemia using MOC and SF OAE measures described in section 2.4.

SF OAE amplitude was a better predictor of older age for lower probe levels. ROC area-under-the-curve (AUC) was used as the "goodness-of-prediction" metric and as shown in Figure 64 below, a probe level of 55 dB SPL and a suppressor level of 70 was optimal for predicting older age, and that higher probe levels did not do much better than chance in predicting older age.

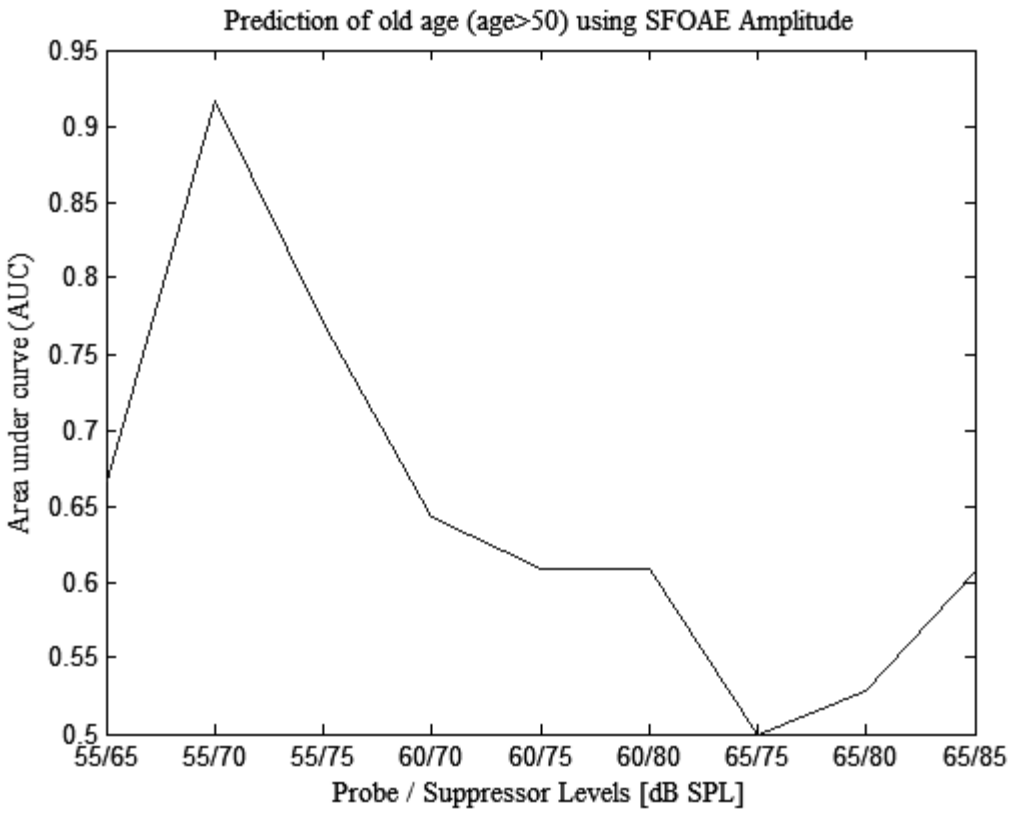

Figure 64: Prediction accuracy for predicting older age based on SFOAE amplitude was significantly better at lower probe levels.

The best probe/suppressor combination of 55/70 dB SPL was selected based on the fact that this probe/suppressor level combination yielded the highest AUC. It was at this probe/suppressor level that the leave-one-out cross-validation was performed. Within this cross-validation, the logistical regression prediction model was trained only on a subset of the data, excluding one of the subject's SF OAE results. Prediction was done on the excluded data. Then a different subjects' data was left out during training and prediction was done on this new subjects' left-out data. Therefore, prediction results are only based on data predicted on model data that was not used in the training. Results from this leave-one-out cross validation ROC analysis are shown in Figure 65 below.

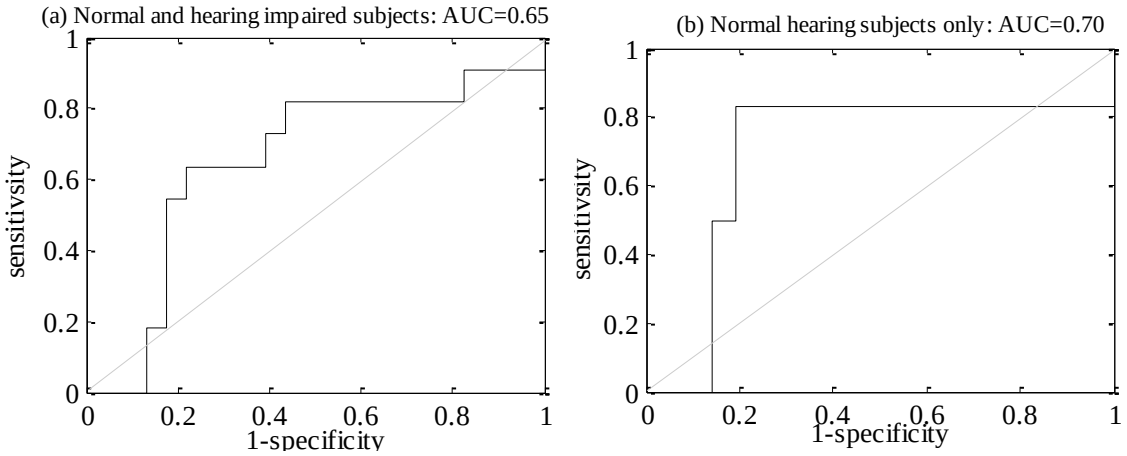

Figure 65: Leave-one-out ROC analysis for predicting older age (>50 years old) using SF OAE amplitude at a probe level of 55 dB SPL and a suppressor level of 70 dB SPL. Results in (a) are for normal hearing (pure-tone threshold <=25) and hearing impaired subjects (N-young = 23, N-old=11). Results in (b) are for normal hearing subjects only. Because of this, the N is smaller in b (N-young=21, N-old=6).

To determine whether SF OAE amplitude is a good predictor of age independent of hearing, we controlled for changes in hearing impairment. Therefore, the ROC analysis was repeated only for subjects with good hearing as defined as pure-tone-thresholds less than or equal to 25 dB HL. As is shown in Figure 65 (b) above, SF OAE amplitude was still able to predict older age for normal-hearing subjects at this probe / suppressor level.

4.3.5. Age within auditory model

In this chapter, we have shown that aging, independent of hearing impairment, has a statistically significant impact on SF OAE amplitude. Older subjects on average had smaller SF OAE amplitudes than younger subjects. There did not appear to be a statistically significant effect of age on SF OAE latency. The effect of aging on SF OAE amplitude ($\Delta V_{SF\ OAE}$(age)) as shown in Figure 60 is summarized below in Table 14.

Probe	Suppressor	Young	Old	Difference ($\Delta V_{SF\ OAE}$(age)
55	65	15.6	12.5	3.1
	70	16	12.7	3.3
	75	16.7	13	3.7
60	70	15.7	14.1	1.6
	75	17.7	16.4	1.3
	80	19.5	18	1.5
65	75	18.2	17.6	0.6
	80	21.1	19.4	1.7
	85	22.3	21.6	0.7

Table 14: Influence of older age on SF OAE amplitude for different probe / suppressor combinations. It is evident from this that the impact of aging is larger and more significant at lower probe / suppressor levels, indicating that it may be the cochlear amplifier that is being impacted by aging.

Figure 66 below is the Ferry & Meddis model (2007) with a box showing how aging could be represented within the model as acting on the cochlear amplifier.

DRNL filter bank with extensions including age effects on cochlear amplifier gain

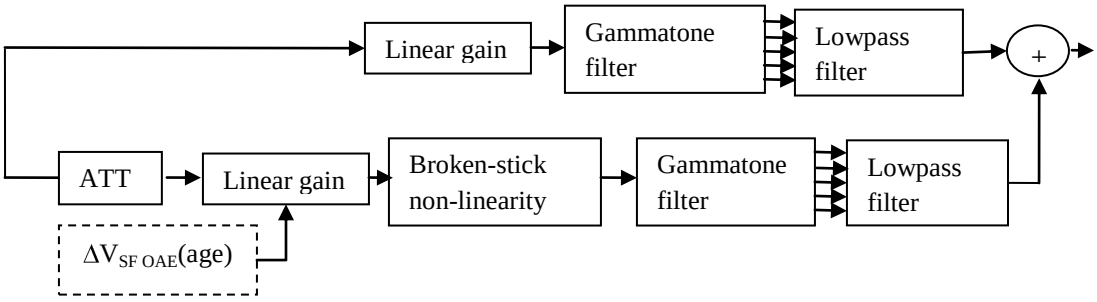

Figure 66: Ferry and Meddis model (2007) shown with impact of aging, $\Delta V_{SF\ OAE}$(age), on the cochlear amplifer.

The fact that aging appeared to impact the SF OAE amplitude less at higher probe / suppressor levels (the region where the cochlear amplifier is fully saturated), indicates that aging may be affecting the cochlear amplifier and therefore the nonlinear gain path within the model. The statistical model results presented in Table 13 show how probe, suppressor level, and aging can be used to estimate the influence of stimulus conditions and aging on cochlear gain.

4.4. Discussion

In this work, we have presented amplitude and latency estimates from SF OAE waveforms using time-domain methods. We have presented a new method for estimating amplitude and latency of SF OAE waveforms based on a curve-fit methodology. SF OAE amplitude estimates of the curve-fit methodology are in agreement with the more traditional FFT window-based estimates of SF OAE amplitude. Estimates of both SF OAE amplitude and latency acquired from young, normal hearing subjects are in agreement with prior estimates of SF OAE latencies measured using onset delays (Konrad-Martin & Keefe, 2005) well as SF OAE group delays (Shera & Guinan, 2003).

We have presented new data that clearly shows and quantifies how both probe and suppressor level cause increases in SF OAE amplitude as shown in Figure 58. We expect SF OAE amplitude to increase with probe levels in general because a larger acoustical input to the ear generates a larger basilar membrane displacement which leads to a larger reflected waveform directed back towards the stapes. A larger suppressor is also expected to yield larger SF OAE amplitudes so long as the SF OAE is not being fully suppressed. Once the SF OAE has been fully suppressed, further increases in suppressor level should no longer generate increases in SF OAE amplitude. Full suppression of SF OAE amplitudes is apparent at the lower probe levels (40-55 dB SPL). For probe levels of 40 and 45 in Figure 58, a change in suppressor level from 10 dB to 20 dB greater than the probe level does not have an overall larger effect on the SF OAE amplitude. This is likely due to the fact that the SF OAE is being fully suppressed by the lowest suppressor level. For probe levels of 50 and 55, larger suppressor levels have a larger effect on the SF OAE amplitude. For the higher probe levels (60 and 65), the suppressor shows a strong correlation with higher SF OAE amplitudes and complete suppression does not appear to occur, even at the largest suppressor level. This could be either because these probe levels generate such a large SF OAE that it requires a suppressor level greater than 20 dB above the probe level to

fully suppress it. Alternatively, it is possible that suppressor levels above 70 dB SPL generate system distortion such that the SF OAE amplitude measurement is contaminated. In a B&K 4157 coupler we measured a larger system distortion for larger probe levels. However, part of our acceptance criteria for a valid SF OAE was that the SF OAE amplitude exceeded that of the system distortion measured in a coupler. For this reason, we are willing to consider the SF OAEs valid at the higher probe levels. This is important for the remainder of the analysis because many of the older subjects only had valid SF OAEs at higher probe levels.

Our results also show a dependence of probe and suppressor level on SF OAE latencies. Figure 59 displays how SF OAE latency is reduced for larger probe levels, and increases for higher suppressor levels. Schairer et al. (2006) showed that SF OAE latency decreased by a factor of about 50% from the smallest probe level (40 dB SPL) to the largest probe level (70 dB SPL). Ruggero et al. (1997) showed in chinchillas that SF OAE latencies decreased by approximately 60% for the lowest probe level (10 dB SPL) vs. the largest probe level (90 dB SPL). These results are nearly identical to our results shown in Figure 59, in which an approximate 50% decrease in latency is observed for 65 dB SPL probes relative to 40 dB SPL probes making the results consistent with these earlier findings.

It is interesting that SF OAE latency tends to decrease with increasing probe levels, but increase with increasing suppressor levels. To understand why we expect SF OAE latency to decrease with increasing probe levels, it is important to understand how basilar membrane mechanics change with probe levels, since SF OAEs are expected to be related to basilar membrane mechanics. At low probe levels, the traveling wave on the basilar membrane is sharp and peaks at the characteristic frequency. As probe levels increase, the traveling wave on the basilar membrane gets broader and shifts apically (Schairer, Ellison, Fitzpatrick, & Keefe, 2006) Recordings made by Rhode and Cooper (Rhode & Cooper, 1996) in the chinchilla showed that phase delay changed very little at the characteristic frequency location, but increased basal to the

CF location and decreased apical to the CF location. Since higher stimulus levels cause a broadening and an apical shift on the basilar membrane, a shorter group delay or SF OAE latency is to be expected. Lineton and Lutman (2003, b) showed that there is a difference between how self-suppression vs. two-tone suppression impacts SF OAE latency. Self-suppressed responses were evoked using suppressor tones (f2) with frequencies very close in frequency to the probe (f1) tone (f2=f1+16 Hz). Two-tone suppressed responses were evoked using suppressor frequencies that were significantly further from the probe tone (f2=1.3*f1). Lineton and Lutman showed both theoretically (2003, a) using Zweig and Shera's cochlear model (Zweig & Shera, 1995) and experimentally (2003, b) that self-suppressed spectral period of the SF OAE increased with increasing suppressor level and that the two-tone evoked SF OAE spectral period decreased with increasing suppressor level. The spectral period is inversely related to SF OAE latency. In our experiment, higher probe levels would lead to effects comparable to the self-suppression experiments described by Lineton and Lutman, while higher suppressor levels would lead to effects comparable to two-tone suppression. And indeed, this is what our results have shown – that for higher probe levels, self-suppression causes an apical shift in basilar membrane motion which leads to a faster group delay and a shorter latency as shown in Figure 59. Likewise, higher suppression levels in our experiment lead to what Lineton and Lutman term a "localized narrowing" of the basilar membrane traveling wave which thereby leads to longer group delays. While Lineton and Lutman showed this phenomenon in terms of SF OAE spectral period, results shown in this dissertation confirm their results and extend them by being the first to directly show how increased SF OAE suppressor levels cause longer SF OAE latencies.

Effects of aging on SF OAE amplitudes were observed and found to be statistically significant, both by performing standard t-tests, ANOVA's, and mixed effects leave-one-out cross-validated model fits. The effect of aging on amplitude was most significant for the lower probe level (55 dB SPL). This is apparent in Figure 60 as the difference in SF OAE amplitude

between older and younger subject decreases with larger probe levels. It is also apparent in Figure 64, which shows that SF OAE amplitude was a very accurate predictor of older age at the lower probe levels. This result may be due to the fact that lower level stimuli force the cochlear to respond within the active region of the cochlear amplifier. As the stimuli increase, the cochlear amplifier becomes saturated. And it may be that the cochlear amplifier mechanism is what is changing with age. If this is the case, then to capture age-related effects, it is important for the cochlear amplifier to be within the active region, where it is responding to lower level stimuli.

Unlike SF OAE amplitudes, latencies showed a slight increase in older subjects, but the change was not statistically significant. This is an interesting finding because it suggests that cochlear tuning, which is hypothesized to be directly related to SF OAE latency (Zweig & Shera, 1995), is not something that changes with aging. OAE latencies have been shown in limited studies to change with noise-induced hearing impairment (Engdahl & Kemp, 1996). Only one aging study is known to have examined OAE latency and aging. Ramotowski and Kimberley showed a slight (0.2-0.3 ms) increase in OAE latency in older adults, and this was a comparable amount of increase in SF OAE latency that we observed (0.2 ms increase in latency for older adults). If aging was negatively impacting the tuning of the cochlea, we would expect a shorter latency as opposed to a slightly longer one. Furthermore, the fact that such a small change was observed (and in our study, statistically insignificant) suggests that age-related effects on the auditory system may be primarily related to changes in the endocochlear potential (EP) or the middle ear, but not specific to hair cell functionality or basilar membrane motion. A small age-related reduction in the EP would certainly lead to reduction in OAE amplitudes, but may not necessarily lead to changes in the cochlear turning. Based on the results obtained in this study and presented in this chapter, we are lead to conclude that age-related hearing changes have more to do with changes in the middle ear or with the EP, and less to do with the tuning of the basilar membrane. We observe systematic and statistically significant drops in SF OAE amplitudes in

older subjects which could be due to a reduced EP or alternatively changes in the middle ear. But we observed no change in cochlear tuning as measured by SF OAE latency, which suggests that the motion of the basilar membrane and possibly the function of outer hair cells are not directly impacted by the aging process.

The influence of aging on SFOAE amplitudes has been demonstrated in this chapter. We do not expect aging effects to directly impact the results acquired during the glucose and attention experiments described in chapters 2 and 3, respectively, because those experiments were measuring acute changes to OAEs that occurred only during the experiment. However, we might expect that the OAEs of the older subjects could be lower in amplitude than those of the younger subjects. Shown below in Figure 67 is the distribution of subjects' age for results from the glucose tolerance tests in chapter 2.

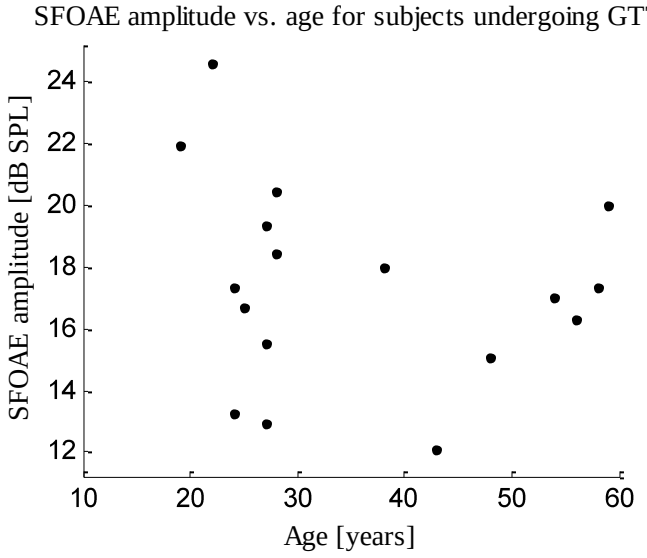

Figure 67: SF OAE amplitude vs. age for subjects tested in the glucose tolerance experiments in chapter 2.

The mean SF OAE amplitude was 18.1 dB SPL for young subjects (<50 years) and was 16.3 dB SPL for older subjects tested in the glucose tolerance testing experiments. The difference in the two groups was not statistically significant, most likely because of the small number of subjects

tested. In the next chapter, we present a discussion and a table (Table 15) that summarizes how glucose, attention, and aging all influence OAEs.

Chapter 5

5. Summary and Conclusions

In this dissertation we have presented results from three primary studies that demonstrate how OAEs and the MOC efferent control pathway correlate and also predict with reliable accuracy specific physiologic changes including (1) blood glucose levels in diabetic patients, (2) focused attention during an active auditory listening task, and finally (3) age related hearing changes. Listed below is a summary of the contributions for each of the three studies presented in this dissertation.

- We have presented results that show that the MOC inhibition amplitude of OAEs in diabetes patients increases in a consistent, systematic, and repeatable way during hyperglycemia.
- We have proposed a biological mechanism explaining why increases in MOC and OAE amplitude were observed during hyperglycemia. The mechanism is based on (i) metabolic influences of blood sugar on the endocochlear potential and cochlear microphonics and (ii) effects of blood sugar on ATP-sensitive ion channels.
- A new method has been presented for predicting hyperglycemia using metrics from MOC inhibition amplitudes and phase waveforms that is called Hyperglycemic Risk Analysis (HRA).
- HRA can be extended to include mixed effects accounting for subject variability; prediction accuracy using leave-one-out cross-validated receiver operating characteristic (ROC) analysis could yield 83% accuracy with 23% false positive rates and an area under the ROC curve of 0.85.
- Prediction of continuous blood glucose is possible, although accuracy is generally less accurate than current off-the-shelf enzyme-based sensors. Clarke error grid analysis on

our methods yields a clinical accuracy rate of 90.2% of data within the A+B region and an r^2 of 0.31 ($p<0.01$). However, only 32% of data fell within the A region and the relative error was high - MARD of 37.2%

- Based on results from the attention correlation study, we have presented further support for the theory that the auditory cortex mediates the activation of the MOC. MOC amplitudes were shown to increase during active attention to an auditory detection task relative to the passive listening condition.
- We have presented results that demonstrate an aging effect on SF OAE amplitudes that is independent of hearing functionality. SF OAE amplitudes in older adults are smaller than in younger adults. No change in SF OAE latency was observed, indicating that tuning (a measure hypothesized to be proportional to OAE latency), is not something affected by aging.
- We have presented a new model that demonstrates in normal hearing subjects how SF OAE amplitude and latency change relative to stimulus levels (probe and suppressor).

In the remainder of this chapter, we will discuss the relevance of the findings and also we will discuss how results from these three studies can be integrated and interpreted. Future work is discussed, several new experiments are proposed, and another future extension of the cochlear model is proposed.

5.1. Relevance of the findings

The results presented in this dissertation are important from a basic science perspective, a clinical applications perspective, and also from a technology and development perspective. The primary finding of this body of work is that elevated blood glucose levels are shown to cause an increase in SF OAE amplitudes and also an increase in the MOC inhibition of SF OAEs in diabetic subjects. From a basic hearing science perspective, this finding is important because it provides support to our understanding that glucose is an important regulator of metabolism within

the ear and that it may also be regulating the auditory system as a neurotransmitter after it has been converted into ATP. The effect of glucose on metabolism and ATP on cochlear neurotransmission has been shown in animal experiments, but the effect has not previously been observed in humans. From a clinical applications perspective, it is important to understand how OAEs change under different glucose levels. OAEs are currently used clinically to diagnose hearing impairment, and if there are changes in OAE amplitudes during hyperglycemia, these need to be understood and factored into clinical judgments that are based on OAE measures. For example, when testing a diabetes patient's hearing, it may be important to also test their glucose levels prior to a hearing test to account for variability in OAE measurements taken. Finally, from a technology development perspective, this primary finding is important because it suggests that hyperglycemia may be an event that can be predicted noninvasively using simple off-the-shelf audio electronics. And if the methods can be improved such that the methods described can match the prediction accuracy of commercial invasive glucose monitoring methods shown in Table 6, continuous glucose monitoring using such a noninvasive, portable auditory test system may be possible. Prior to this being the case, however, effort must be put into improving the speed, accuracy, and reliability of the measurement system. And tests on hypoglycemia must be done to verify that the change in MOC inhibition and SF OAE amplitude can be observed when there is a lack of available glucose in a patient's blood. If an accurate and reliable noninvasive blood glucose monitor can be built using simple off-the-shelf auditory equipment, it would significantly reduce the cost of blood sugar monitoring and make it easier and less painful for diabetic subjects to monitor their sugar levels. This could then result in better control of the disease, fewer life-threatening hypoglycemic episodes for diabetic patients, and a reduction in damage caused by long-term exposure to elevated sugar levels including retinopathy, neuropathy, and loss of limbs.

A secondary finding of this work is that the MOC inhibition amplitude, on average, increases when a test subject actively attends to an auditory attention task as compared with the passive listening condition. This finding is relevant from both a basic hearing science perspective and a clinical applications perspective. Prior hearing science research has not confirmed whether the MOC inhibition of the cochlear amplifier can be actively controlled by the auditory cortex and findings from other studies have been mixed (Maison, Christophe, & Collet, 2001; de Boer & Thornton, 2007; Harkrider & Bowers, 2009; Ferber-Viart, Duclaux, Collet, & Guyonnard, 1995). Results from this work support the work done by Maison et al. (2001) and we have provided an explanation for why results from other groups have been different. The clinical relevance of showing that the MOC can be cortically controlled is significant. The MOC is believed to be important for helping people better understand speech in noisy environments. The MOC has been shown to be a predictor of patients' ability to improve their performance in speech-in-noise testing as a result of training (de Boer & Thornton, 2008). If hearing impaired patients can be trained to more directly use their MOC to improve their ability to understand speech in noise, this may be a strong indication for developing a training regimen based on improving facility of MOC control.

The other major finding in this study is that aging affects SF OAE amplitude but not latency. Findings from this study are relevant from both a basic science and a clinical applications perspective. There has not been prior work investigating the effect of aging on SF OAE amplitude or latency. Therefore results presented here are new and relevant to our basic understanding of how aging affects the auditory system. The specific finding that aging affects SF OAE amplitude but not latency implies that age-related changes to the auditory system may influence the auditory system (possibly through changes in the middle ear or the endocochlear potential), but not the tuning within the ear since OAE latency is proposed to be directly related to tuning (Shera & Guinan, 2003). From a clinical applications perspective, it is important to

understand aging effects on the OAE since OAEs are used for diagnosis of hearing impairment. It may be necessary to include an age-specific correction factor to OAE measures. Results given in Table 14 provide the means to develop such a correction factor.

5.2. Integrating and interpreting glucose, attention, and aging results

It can be challenging to interpret which effects on the MOC may be due to attention vs. elevated blood sugar levels since both conditions showed a change in the MOC inhibition amplitude. In chapter 2 results were presented that showed how MOC inhibition amplitudes in diabetic subjects consistently increased during hyperglycemia. In chapter 3, we presented results that showed how MOC inhibition amplitudes also increased during a focused attention task. How can we distinguish changes in MOC inhibition amplitude presumed to be caused by hyperglycemia from potential changes in the subjects' attention during the glucose tolerance test? Recall from chapter 2 that for the diabetic subjects, we controlled for focused attention by requiring the subjects to watch silent movies (reading subtitles while watching the film) during the entire glucose tolerance test. The reason why we did this was to encourage the subjects to disregard or ignore the audio stimuli used to evoke the OAEs and MOC inhibition during the testing and instead focus on the movie. While we cannot be sure that the subjects were not still attending to the audio during the testing period, this requirement within the procedure ensured a certain qualitative level of control over the subjects' attention. The quantitative evidence that the change in MOC inhibition during hyperglycemia was indeed due to sugar levels and not changes in the auditory attention were based on results presented in section 2.3.5. In this section, results are presented from three subjects who underwent the same glucose tolerance test experiment without consuming the sugar. The fact that their MOC did not increase statistically during the second half of the control experiment (the period when subjects would usually have elevated sugar levels) relative to the beginning of the experiment suggests that there was no time-based change in MOC inhibition caused by changes in focused attention to the auditory stimuli.

The testing on the non-diabetics was done earlier in the study, and we did not have the foresight to include control measures for auditory or visual attention during those initial tests. For this reason, while most of the non-diabetic subjects watched silent movies during the GTT, some of the subjects did not, and it was not a variable that we recorded during the testing. For this reason, results from the non-diabetic testing may be affected by changes in auditory attention during the glucose tolerance test. However, it is important to note, that the rise in MOC inhibition during hyperglycemia was significant only for the diabetes subjects. Therefore, results from the non-diabetes subjects, although interesting, are certainly less relevant to this dissertation than results from the better-controlled experiment on diabetes subjects.

While attention has been maintained adequately during the glucose tolerance testing, at least for the diabetic subjects evaluated, a question remains regarding whether blood glucose changes during the attention tests could have occurred, thereby affecting the results. To control for this, all but three of the subjects tested were non-diabetic subjects, so their glucose would be expected to remain constant and well below 150 mg/dL during the testing. The diabetic subjects tested their sugar levels before and after the testing, and it was found that their levels remained lower than 150 mg/dL during the attention test, which is lower than the 160 mg/dL glucose level we defined as "hyperglycemic" for diabetic patients. Therefore, all subjects performed the attention study under what we termed as "normal glycemia" conditions with blood glucose below 150 mg/dL.

Aging effects did not play a role in either the glucose experiments discussed in chapter 2 or the attention experiments discussed in chapter 3. The glucose experiments and the attention experiments involved acute changes in glucose or attention, respectively. And therefore age did not play a role in the outcomes of the acute changes in the OAEs or the MOC activation.

5.2.1. Extending auditory models to include glucose, attention and age

In chapter 1, the Ferry and Meddis model (2007) was discussed and we showed how glucose and attention affects on the MOC as well as age effects on the SF OAE amplitude (see Figure 13) could be represented within the model. To view how the MOC inhibition amplitude changes with both glucose and with attention, the changes in the MOC inhibition amplitude distributions are plotted below during normal glycemia vs. hyperglycemia (Figure 68) and also during passive attention vs. active attention tasks (Figure 69).

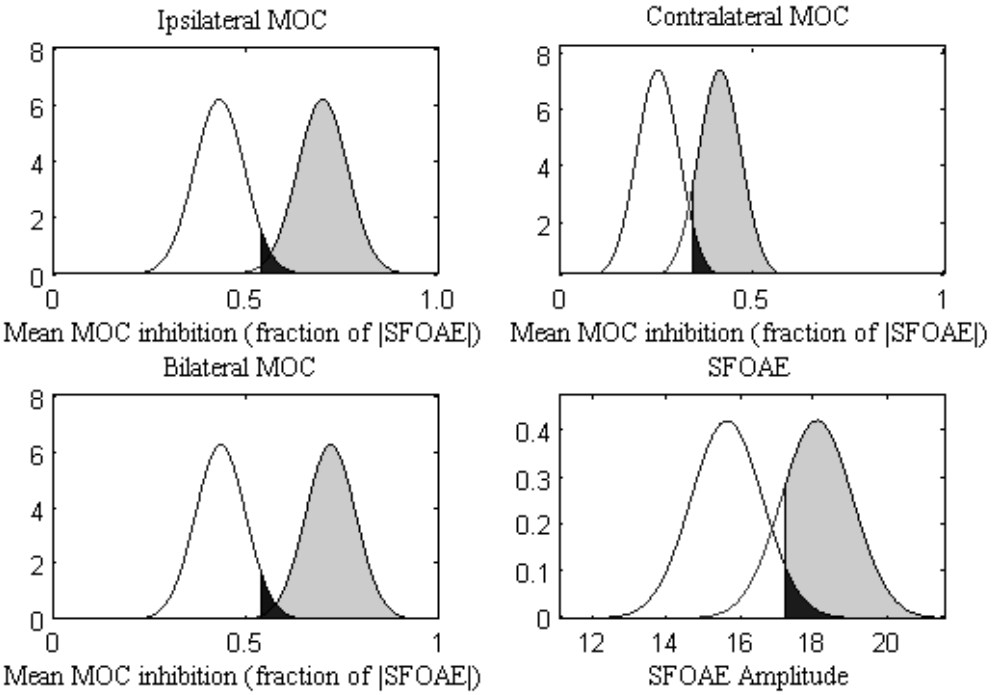

Figure 68: Distribution of MOC inhibition and SF OAE amplitudes during normal (white) and hyperglycemia (shaded). We show the power of the difference in the distributions since p-values are shown earlier in Figure 26.

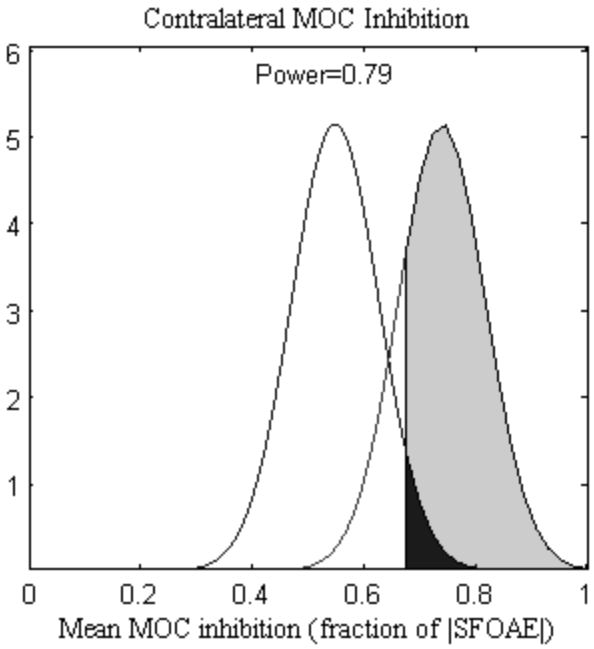

Figure 69: Distribution of MOC inhibition amplitude during passive (white) and active (shaded) attention.

It is clear from the figures above that hyperglycemia and active attention affect the MOC in similar ways and at nearly equivalent magnitudes. This is why the control aspect of the experiments were so critical to determining whether effects measured during the experiments were truly due to attention compared with sugar levels.

The model parameters that fit into the extension of the Ferry and Meddis model are given in Table 15 below and are taken from the results shown earlier in Table 7, Table 9, and Table 14.

Model Param	Physiologic event	Observed measure	Change in measure
ΔV(g)	Hyperglycemia	Ipsilateral MOC	0.26 (fraction of |SF OAE|)
	Hyperglycemia	Contralateral MOC	0.16 (fraction of |SF OAE|)
	Hyperglycemia	Bilateral MOC	0.28 (fraction of |SF OAE|)
	Hyperglycemia	SF OAE amplitude	2.42 dB SPL
ΔV(a)	Active attention	Contralateral MOC	0.19 (fraction of |SF OAE|)
ΔV(age)	Old age	SF OAE amplitude	-3.3 dB (55 dB Probe, 70 dB Suppressor)

Table 15: Comparison of changes observed in MOC and SF OAE with respect to blood glucose, attention, and aging.

5.3. Attention as a confounding factor to glucose estimation

From the results shown in Table 15, it is clear that if MOC inhibition of OAEs is ever to be used clinically to monitor blood sugar, it will be imperative to devise a way to ensure that the subject is always paying the same amount of attention during the measurements. One method that could be used to ensure such consistency would be to require the subject to do a task during the measurement. One simple task that could be done to ensure a consistent level of attention would be to require the subject to count the number of sound presentations done during a test. Once the measurement is made, the protocol would require the subject to enter the correct number of sound presentations they had heard. If they are correct, then the glucose reading would be presented to the subject. If they were incorrect, the measurement would need to be repeated until the subject answered correctly.

5.4. Other confounding factors influencing future use of MOC and OAEs in diagnosis and rehabilitation

There are many drugs that affect OAEs. Aspirin is known to cause reduction on DP OAE amplitudes (Wier, Pasanen, & McFadden, 1988; Abdala, 2005) and to influence cochlear mechanical tuning (Brown, Williams, & Gaskill, 1993). Caffeine and ryanodine have been shown to reversibly reduce compound action potentials (Bobbin, 2002) . Caffeine is known to influence the release of calcium, which has been shown to be a necessary component of ATP-induced inhibition effects of OAE amplitudes (Ashmore & Ohmori, 1990; Nakagawa, Akaike, Kimitsuki, Komune, & Arima, 1990; Dulon, Mollard, & Aran, 1991; Housley, et al., 1999; Yu & Hong-Bo, 2008). Drugs used to treat other ailments are also known to affect hearing functionality and therefore OAE measurements. For example, chlorpromazine, a common treatment for schizophrenia, is known to increase DP OAE thresholds and inhibit hearing (Oghalai, 2004). And cisplatin, a drug used in chemotherapy treatment, is known to impair hearing and cause reduction in OAE amplitudes (Zorowka, Schmitt, & Gutjahr, 1993; Dille, McMillan, Reavis, Jacobs, Fausti,

& Konrad-Martin, 2010). In our experiments, we did not permit subjects to consume aspirin or caffeine these during or before testing, and we ensured that subjects were not taking any ototoxic medications that inhibited hearing functionality. In this way, these substances did not influence our results. However, if OAEs and the MOC are to be measured periodically throughout a person's daily life as would be done with a noninvasive blood glucose monitor, it is important to better understand how different drugs and foods could potentially interfere with the measurements taken. A thorough analysis would be necessary to assess how these substances affect the results. And in future research studies, it would be important to develop a portable OAE/MOC measurement system that could be used to make measurements throughout a person's day as they consume different substances to determine whether any substances are confounding results.

5.5. Concluding remarks and future directions

The work presented within this dissertation opens the door for several important future investigations. The first future study proposed would be one which would help to differentiate the role that blood sugar plays on ATP sensitive ion channels as compared with its influence on the endocochlear potential. It would be interesting to better understand whether these two competing influences could be teased apart, so that the influence of blood sugar on each of these processes could be more directly measured. And it would also be interesting to better understand how the MOC mediates these effects through the release of calcium ions. This experiment would likely involve an animal model whereby the endocochlear potential could be maintained constant (possibly through the use of drugs) during infusion of blood glucose into the cochlear perilymph and endolymph. Perhaps calcium ion concentration could be monitored simultaneously within the endolymph while OAEs are recorded during MOC elicitation. Such an experiment would help to confirm our hypothesis that the mechanism leading to larger MOC activation during hyperglycemia is caused by the influence of calcium-dependent ATP sensitive ion channels,

which could be modulated by the MOC activation through acetylcholine release. Parts of this experiment have previously been performed (Yu & Hong-Bo, 2008; Kujawa, Fallon, & Bobbin, 1994b; Bobbin & Thompson, 1978; Munoz & Thorne, 1995; Munoz, Thorne, & Housley, 1999; Sueta, Paki, Everett, & Robertson, 2003; Kujawa, Erostegui, Fallon, Crist, & Bobbin, 1994a; Skellett, Chen, Fallon, Nenov, & Bobbin, 1997; Ashmore & Ohmori, 1990), but the entire mechanism has not been examined including the role of the MOC in this process. It would also be of interest to explore the role of hypoglycemia as well as hyperglycemia on these two processes.

Variation of OAEs and the MOC during hypoglycemia was not investigated in the experiments presented within this dissertation. In a future study, a glucose clamp procedure could be done whereby a human test subject's blood sugar is maintained at a constant level through the use of intravenous insulin and glucose infusions while OAE and MOC measurements are done. Such a glucose clamp experiment has been done by other researchers investigating auditory functionality under hypoglycemic and hyperglycemic conditions (Sasso, et al., 1999) and this can enable measurements to be made during severe hypoglycemia within a safe environment under a medical doctor's observation. Running the experiment as a glucose clamp also enables many more measurements to be taken at a single glucose level. During the testing described in this dissertation, the subject's glucose level changed very rapidly after they consumed the sugar. It was not possible to get a large number of OAE and MOC measurements at a single glucose level. The glucose clamp experiment would enable a larger sample size of measurements and would help to ensure the accuracy of the measurements acquired. And a glucose clamp experiment is the only way to measure the OAE and the MOC under severe hypoglycemic conditions.

Another future area of work is in the area of exploring how MOC inhibition might change during hyperglycemia at different locations along the basilar membrane. By observing

MOC-induced inhibition effects at different audio frequencies, we might observe different behavior and perhaps more consistent results during hyperglycemia. A future experiment could entail using a swept tone to evoke the SF OAE as was done by Choi et al (2008). Choi et al. swept a tone from 550 to 1450 Hz thereby evoking OAEs continuously along the corresponding region of the basilar membrane. By eliciting the MOC during the swept tone, we could observe whether averaging MOC inhibition amplitudes across multiple frequencies would improve the signal-to-noise ratio of the measurement and if that would improve the correlation of blood glucose with MOC inhibition. It would also be of interest whether different regions of the basilar membrane might be more responsive to changes in blood glucose levels or attention.

There is also future work in the area of focused attention including more experiments investigating how cue-dependent attention bandwidths can be estimated physiologically using MOC or other SF OAE metrics. In the attention tests described in chapter 3, the SF OAE was measured spectrally distant from the probe and cue. This may have prevented us from observing an effect of cue on MOC inhibition. This would especially be true if the cue has a release-from inhibition influence on the MOC only within the critical bandwidth of the frequency of the cue. It would also be important to further investigate whether having the probe finding task and the SF OAE measurement in the same ear might influence results and ultimately reveal an influence of the cue on MOC inhibition.

Another particularly interesting area for future work is furthering the development of the Ferry and Meddis auditory model to include bandwidth dependent activation requirements of MOC inhibition. Such a model extension would account for the timing of MOC activation and the fact that high-level broad-band auditory stimuli tend to activate the MOC as compared with narrow-band stimuli and tones. In the model extension, spectral bands from the "hair cell transduction and adaptation" part of the auditory model are summed and put through a nonlinear gain stage. The summing across multiple frequency bands is used to model the fact that typically

it is broadband stimuli that more easily activates the MOC, so inputs from multiple bands sum together to activate. If the summed input to the threshold stage exceeds a certain level, then the MOC becomes active. Finally, the MOC activation is low-pass filtered to better represent how group nerve responses exhibit a smoothed onset/offset impulse response. The extended model is shown in Figure 70.

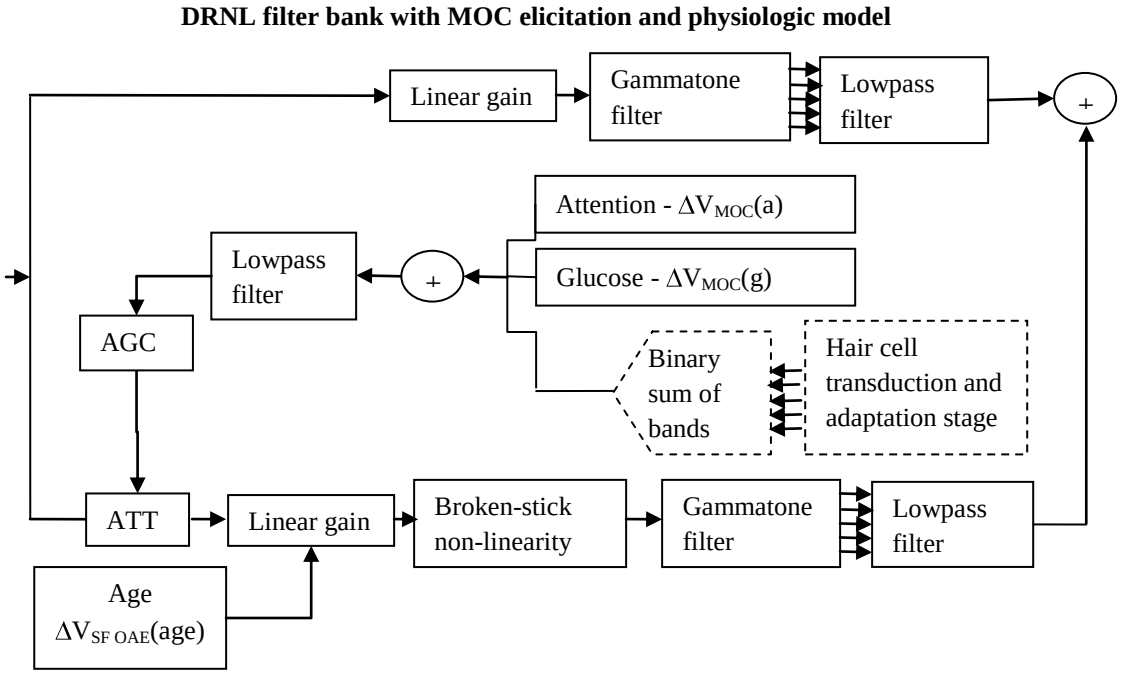

Figure 70: Extension of Ferry and Meddis MOC model (shown in red). The model extension includes effect of broad-band elicitors on MOC activation.

The proposed equivalent feedback circuit model for the extension shown above is shown in Figure 71.

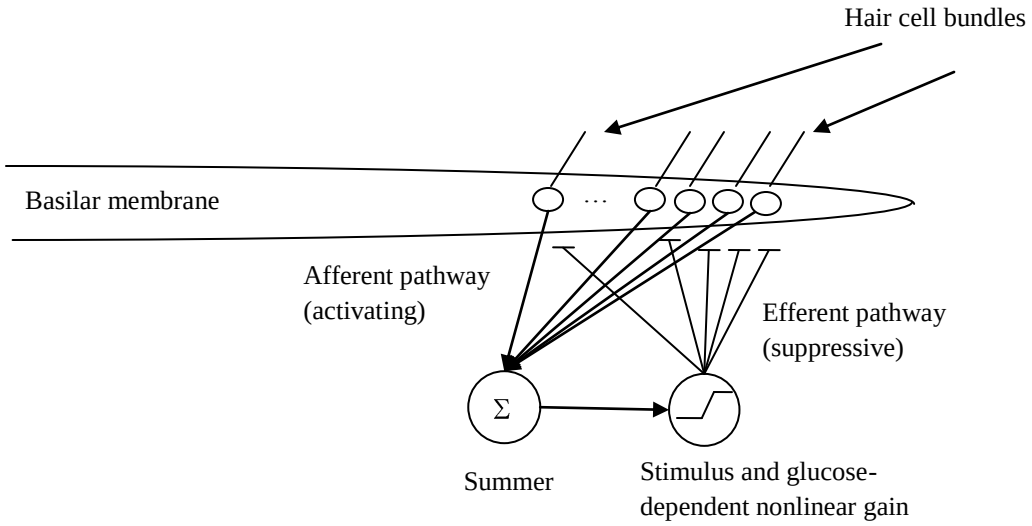

Figure 71: Physiologic neural network interpretation of including a spectrally dependent activation mechanism for initiating the MOC.

In future work, this model extension will be developed, trained and evaluated on different types of audio stimuli. Development of this model is important since work by other groups has shown that MOC activation can improve speech perception both in human speech-in-noise tests (Kumar & Vanaja, 2004; de Boer & Thornton, 2008), and also when using the Ferry and Meddis model in an automated speech recognizer task (Brown, Ferry, & Meddis, 2010). However, current hearing aid algorithms do not utilize the MOC model as a means for improving speech-in-noise performance. A robust and accurate algorithmic implementation of the MOC inhibition could prove to be an important component of future hearing aid algorithms.

In Summary, there are several promising areas of future work that can be based on the foundation research presented in this dissertation. Further investigation is merited for studying the metabolic and neurotransmission influences on the OAEs and the MOC. The observations made in this dissertation should be reproduced, preferably under a glucose clamp experiment that would enable measurements to be made over longer periods of time in the severe hypo and hyperglycemic regions of interest. Improvements can be made in measurement accuracy and an investigation should be done into whether different regions of the basilar membrane provide

complementary or unique information indicating blood glucose and/or attention variability. There should be additional research into the measurement variability of OAEs and MOC as measured within a typical daily living environment. Such a study would help to better understand whether different activities, foods, or drugs cause anomalies in the OAE and MOC readings. Such a "walk-about" study would require the development of a portable OAE measurement system. To ensure a patient's consistent focused auditory attention during measurements, a protocol like the counting task described earlier would need to be further developed and evaluated that would help ensure that the patient's constant concentration. Finally, additional development can be done to ensure that the MOC can be accurately modeled, not just with simple tones and noise, but in a dynamic environment where sounds are changing rapidly, such as with speech. Such an MOC model may prove effective at improving a hearing impaired person's ability to understand speech in noise if used effectively within a hearing aid.

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7. Appendix

The paper below represents early pilot work on this project. It was published in the 30th Annual IEEE Engineering in Medicine and Biology Conference Proceedings, 2008, pp 4704-4707. The results of this pilot project provided the motivation for performing the additional work described in chapter 2 of this dissertation.

On Correlating Otoacoustic Emissions with Blood Glucose Levels

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***Abstract*— The long term objective of this research is to develop a new means for diabetic patients to painlessly and non-invasively monitor their blood glucose levels. We propose a novel method for noninvasive glucose monitoring based on measurement and analysis of otoacoustic emissions (OAEs). OAEs are low-intensity sounds generated by the cochlea in response to acoustic stimuli. Evoking and measuring OAEs is done using a tiny speaker and microphone that fit snugly inside the ear canal. The OAE response can be partially masked or reduced in amplitude by presenting competing acoustic stimuli contralaterally (opposite ear), ipsilaterally (same ear), or both. This masking effect is caused by activation of neural efferent pathways from the brain. Neural effects, including evoked responses such as auditory brainstem responses and axonal transmission latencies, are known to correlate with glucose. This suggests that masked OAEs may correlate with glucose since masking is a result of neural activity, and neural activity is affected by glucose levels. Prior to our research, no studies have investigated the correlation of *masked* OAEs with blood glucose. In this paper we present our preliminary findings, including experimental results that suggest a correlation with blood glucose levels.**

Funding for this research from NIH/NIDDK 1R21 DK079283-01A1 and VA RR&D grant C2659C

INTRODUCTION

CURRENT invasive methods of monitoring blood glucose levels in diabetic patients involve painful finger sticks. Many diabetic patients fail to actively manage their glucose for the primary reasons of finger soreness, pain, inconvenience, and fear of needles [1].

Researchers have been searching without success for ways to measure blood glucose noninvasively for many years [2]. Approaches include using near infrared technology to noninvasively obtain optical signatures known to correlate with glucose levels, or using ionophoresis, whereby electrical currents are applied to the surface of the skin to extract and analyze samples of interstitial fluid. Both of these methods pose problems, including inaccuracy, skin irritation, and calibration problems [2].

Our long term goal is to develop a handheld device that diabetic patients and clinicians may use to monitor blood glucose noninvasively using otoacoustic emissions (OAEs). The normal cochlea does not just receive sound; it also produces low-intensity sounds called OAEs [3]. We evoke OAEs by presenting the ear with sounds and measuring the cochlear response using a tiny microphone. The microphone measures the total pressure in the ear, which consists of a larger audio stimulus combined with the much smaller OAE response. The OAE can be extracted by presenting two tones to the ear simultaneously, a probe tone and a suppressor tone, and measuring the amount that the suppressor reduces the amplitude of the cochlear response to the probe. This amount of suppression is an approximation of the OAE amplitude. When the probe and suppressor are close in frequency, this type of OAE is called a stimulus frequency OAE (SF OAE). In our experiments, we do not expect the SF OAE to change directly with glucose levels, but rather we expect efferent nerve responses that modulate the SF OAE to change with glucose.

Glucose affects cortical auditory processing [4] and auditory nerve pathways, including evoked responses and axonal transmission latencies during the hyperglycemic state [5]-[6]. Studies of the auditory brainstem response (ABR) have shown central conduction time delays in diabetic subjects [7], changes during insulin-induced hypoglycemia [8], and changes during glucose clamp induced hyperglycemia conditions [7]. De Feo et al. [9] demonstrated an inverse relationship between ABR wave latencies and plasma glucose.

While the OAE is not itself a neural response like the ABR, OAE amplitudes are reduced or *masked* by activation of efferent nerve fibers that terminate on sensory cells that generate OAEs. These fibers are

activated when a sound such as broadband noise is presented to the contralateral or ipsilateral ear, or both [10]. Masking is a result of a neural modulation of the OAE response [10]. The primary nerve bundle believed to be responsible for this masking effect is the medial olivocochlear (MOC) bundle. Measurements of MOC masking can often be confounded by middle ear muscle (MEM) reflex, another neural response capable of having a masking effect on an OAE [11].

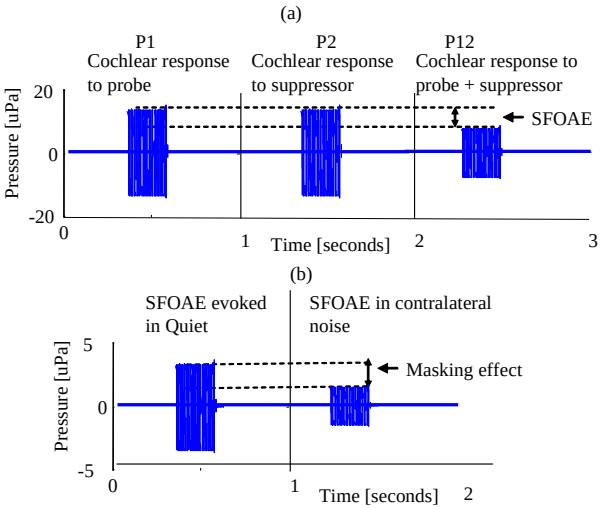

Figure 1: (a) Double-evoked paradigm for evoking and recording SF OAE. (b) SF OAE evoked during quiet and with contralateral noise. Masking effect is

MOC effects on OAEs may be influenced by changes in axonal firing rates, conduction times, and number of nerve firings, all of which may manifest as OAE amplitude and latency changes. This suggests that OAEs masked by contralateral or ipsilateral audio stimuli may correlate with glucose since the masking is mediated by peripheral nerve feedback, and nerve activity is affected by glucose. Therefore, we expect the masked OAE to correlate with blood glucose variation.

Suckfull [12] found that OAE amplitudes correlate with blood glucose levels in rabbits. Using single-frequency tone bursts to evoke OAEs, Suckfull recorded OAE amplitudes in rabbits while infusing their blood serum with 40% glucose and observed decreases in evoked OAE amplitudes in response to the elevated glucose levels. In contrast, Sasso [7] examined OAE amplitudes during hyperglycemia for 10 diabetic and 10 non-diabetic human subjects and found no correlation with glucose levels. Based on current information on osmotic and metabolic mechanisms of glucose perfusion within the cochlea and based on our own preliminary data, we do not expect unmasked OAEs to *strongly* correlate with blood glucose.

Neither Sasso nor Suckfull examined the effects of glucose on an OAE masked by contralateral or ipsilateral audio. There are compelling reasons as argued above why we expect a more pronounced correlation of masked OAE amplitudes and latencies with glucose than with unmasked OAEs. In this paper, we describe methods and present our preliminary findings on correlating blood glucose with OAEs

masked by contralateral and ipsilateral noise.

PROCEDURES

OAE measurements were taken using custom data acquisition software running on a desktop computer. The A/D converter was a CardDeluxe sound card. An Etymotics Research ER2 miniature speaker was used to present audio to the ear and an ER10B+ microphone was used to record OAEs. A custom amplifier was used to amplify the audio before being sent to the A/D. Contralateral audio was presented using a GSI Audiometer. Calibration was done using a B&K 2260 Sound Level Meter.

OAEs were evoked from test subjects while the subjects underwent a glucose tolerance test (GTT). A GTT is a procedure whereby subjects' blood glucose levels are elevated through the consumption of a large quantity of sugar after fasting. The subjects fasted overnight for at least 8 hours. After consumption of the sugar, the subjects' OAEs were recorded while simultaneously monitoring blood glucose every 5 minutes using finger-prick blood draws and a Therasense Freestyle glucose meter.

We performed two preliminary studies to help answer the question of whether temporal and spectral features within a masked OAE correlate with blood glucose levels.

Preliminary Study 1 – Methods for Contralateral OAE Masking

In preliminary study 1, we examined whether an OAE masked with contralateral noise varied in amplitude and latency with respect to blood glucose levels in a non-diabetic subject. SF OAEs were evoked both during contralateral stimulation with broadband noise and without contralateral noise stimulation while the subject underwent a GTT. The SF OAE was evoked using two tones, a 2000 Hz probe tone (S1) and a 1940 Hz suppressor tone (S2). We used a double-evoked (2E) measurement technique that measures a nonlinear residual [13] in Figure 1a. Frequency tones are presented sequentially to the ipsilateral ear every second. The audio stimulus presented in the first one-second interval is the probe while the audio stimulus presented in the next second is the suppressor. The suppressor and the probe are presented simultaneously during the third second of the 3-second triplet stimulus. We will call the cochlear response to the probe P1, the response to the suppressor P2, and the response to both the suppressor and probe played simultaneously P12. When the suppressor and probe are played simultaneously, the cochlear response is reduced in magnitude compared with the sum of the response to the probe and suppressor played independently. This nonlinear reduction in amplitude is the SF OAE

response. The SF OAE is calculated as P1+P2-P12. In the condition where no OAE is evoked, such as in a coupler, this quantity equals zero. The SF OAE was evoked with and without contralateral noise stimulation. The difference in the size of the SF OAE under these two conditions is the contralateral masking (Figure 1b).

We executed two runs using two sets of sound pressure levels (SPL) of the stimuli. In the first run, we presented S1 at 50 dB SPL, S2 at 65 dB SPL, and the contralateral noise at 30 dB HL (hearing level). In the next run, we presented S1 at 60 dB SPL, S2 at 70 dB SPL, and contralateral noise at 40 dB HL. S1 and S2 were presented as 10 ms pulses while the noise was presented continuously.

Preliminary Study 2 – Methods for Ipsilateral OAE Masking

In preliminary study 2, we used a forward-masker audio stimulus in the ipsilateral ear to mask the OAE response and again observed masked OAE amplitude relative to blood glucose levels in 5 type 2 diabetic subjects, one type 1 diabetic subject, and one non-diabetic subject. In the ipsilateral masking stimulus paradigm, we again used the double-evoked OAE stimulus method, with a few differences. In this stimulus paradigm, we did not present contralateral noise. Recall that in the contralateral stimulus paradigm, the masker and the probe overlap in time within the 1-second window. In the forward-masking paradigm, the masker stimulus is presented in the ipsilateral ear immediately before the probe signal in the 3-second stimulus triplet. Presenting the masker immediately before the probe causes the amplitude of the OAE response to the probe to be masked, or smaller in amplitude than when the masker was not presented. We call the cochlear response to the masker P1, the response to the probe when the masker immediately precedes it P2, and the response to the probe when there is no masker preceding it P3. The ipsilateral forward masking effect is calculated as P3-P2 (Figure 2). The forward masking noise was presented as a 400 ms 89 dB SPL pulse. For Type 1 and nondiabetic subject test runs, we used a 100 ms probe consisting of a 1260 Hz tone. For Type 2 5-patient test run, we used a 100 ms probe consisting of a combined tone (250 Hz + 1500 Hz) presented at 74 dB SPL.

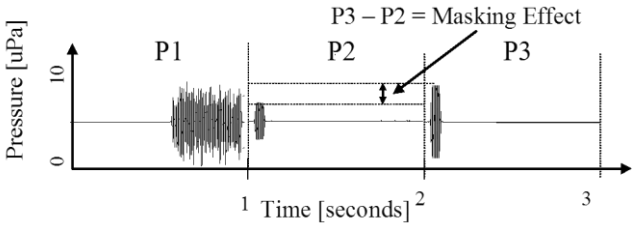

Figure 2: Forward masking OAE recording.

Analysis

All audio stimuli were sampled at 22,050 Hz. Each triplet of 1-second audio was presented 32 times and the nonlinear masking effect was averaged to reduce noise. The averaged result was then passed through a 3516-tap FIR bandpass filter with a 30 Hz bandwidth centered at the probe frequency with a group delay of 80 ms. The envelope of the averaged waveform was obtained using a Hilbert transform to extract amplitude and latency. Latency of the masked response was measured relative to the probe signal. Amplitude and SNR were extracted using the amplitude from the FFT of the masked response taken at the probe frequency. Correlation with blood glucose was determined by fitting a line between the predicted vs. actual blood glucose levels. Prediction was done with multiple linear regression using the amplitude and latency OAE metrics.

RESULTS

Preliminary Study 1 Results: Contralateral Masking

We found a positive correlation existed for amplitudes of the masked SF OAE amplitude for both combinations of masker / probe stimulus parameters tested during contralateral masking. Figure 3 shows that as the subject's blood glucose level rose, so did the SF OAE masked amplitude. This suggests that as glucose levels rise, we are observing less masking taking place, since the amplitude of the SF OAE is increasing. Multiple linear regression using OAE amplitudes during contralateral noise for probe stimulus at 50 dB SPL and 60 dBSPL showed a correlation of 0.766 ($p<0.05$). We did not observe a correlation of *unmasked* SF OAEs with glucose. This is in agreement with prior results [7] and suggests that our hypothesis was correct that it is the efferent pathway that is primarily affected by glucose levels, not the cochlea itself.

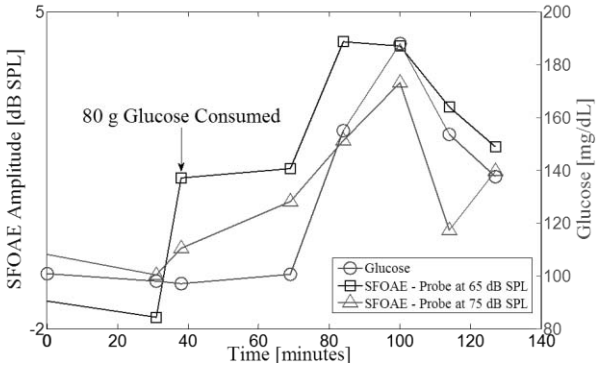

Figure 3: SF OAE amplitude recordings evoked during contralateral noise masking taken during a glucose tolerance test. Subject's glucose (circles) shown. The arrow shows when the subject consumed 80 g of glucose.

Preliminary Study 2 Results: Ipsilateral Masking

Non-diabetic subject results

Results from the forward-masking study also revealed a positive correlation of the masked OAE with glucose levels in the non-diabetic subject. Figure 4 shows the masked OAE amplitude tracking the glucose level. Linear regression resulted in and a correlation of 0.57 ($p<0.05$) between the masked OAE amplitude and the blood glucose levels.

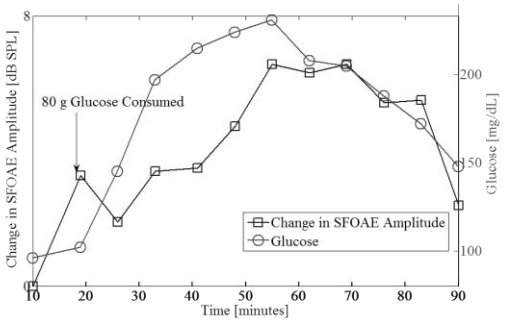

Figure 4: Results from a non-diabetic subject using ipsilateral noise masking during a glucose tolerance test. Masked OAE amplitude increased with increasing glucose levels.

As was observed in the contralateral masking study, the masked OAE amplitude overall appears to increase with increasing blood glucose levels. As the blood glucose falls, the masked OAE amplitude also drops again. The latency of the masked OAE did not appear to correlate with glucose.

Type 1 Diabetic Results

Results from a diabetic subject tested using ipsilateral forward masking were remarkable in that both the masked OAE amplitude and latency were observed to tightly correlate with the subject's blood glucose during the glucose tolerance test. While both amplitude (Figure 5a) and latency (Figure 5b) showed strong correlation, latency was particularly strong ($R2 = 0.89$, $p<0.05$).

Results of 5 Type 2 Diabetics: Ipsilateral Masking

While results from testing the five type 2 diabetic subjects helped to confirm results from our other test runs, the results were less consistent. Correlations between glucose and the individual OAE metrics (amplitude, latency, and SNR) were only observed across two of the five patients. Possible reasons for the inconsistency are discussed below.

DISCUSSION

We have shown that masked OAE amplitudes and latencies correlate with blood glucose in several diabetic and non-diabetic patients tested. However, we have observed inconsistency across test subjects. Of the 5 type-2 diabetic subjects, only 2 of 5 subjects demonstrated a correlation. We believe that the inconsistency in our results is partly due to the fact that the masking effect we observed was not exclusively due to either the middle ear muscle reflex (MEM) or the medial olivocochlear (MOC) reflex nerve pathway. By using high amplitude audio stimuli in the ipsilateral masking study we were able to evoke a repeatable masking effect, however, we also were likely evoking both the MEM and MOC reflexes. It is likely that the MEM overpowered the MOC reflex.

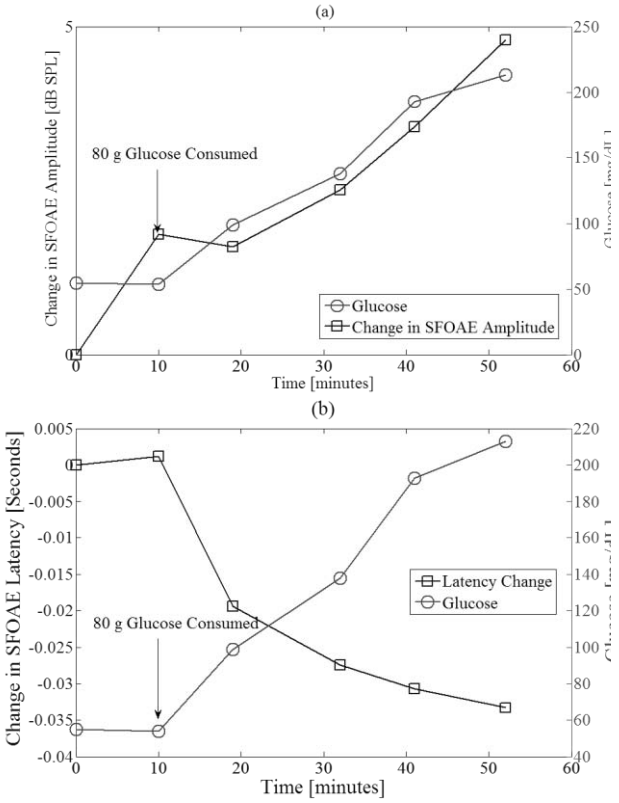

Figure 5: Results from testing a diabetic subject using ipsilateral noise masking during a glucose tolerance test. Masked OAE amplitude (a) increased with increasing glucose levels while latency (b) decreased.

We plan to try additional tests involving a larger number of subjects which will help us determine if an effect is observable due to the MOC reflex alone. We expect that by using both contralateral and ipsilateral noise simultaneously to activate the MOC reflex, we will observe a more robust masking effect using significantly smaller amplitude probe and masking stimulus levels. We will test a variety of additional masking and probe stimuli including lower amplitude tones, chirps, and filtered noise. We also plan to implement tighter control for age and diabetes-related peripheral neuropathy, both of which affect the MOC.

Future plans also include development of algorithms that will be used to accurately predict glucose levels

based on current and prior OAE measurements. A larger test sample and better prediction algorithms will help us further assess whether these methods may ultimately be used for noninvasive blood glucose monitoring.

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