Electrophysiological Assessment of Internal Noise in the Human Visual Pathway

BY

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LIST OF ABBREVIATIONS

fERG	Flicker Electroretinogram
fVEP	Flicker Visual Evoked Potential
pERG	Pattern Electroretinogram
N_{eq}	Equivalent Internal Noise
LAM	Linear Amplifier Model
UIC	University of Illinois at Chicago

SUMMARY

Purpose. Noise is present throughout the visual system, from photoreceptors to visual cortex. The internal noise of the visual system that limits function has been studied for several decades using behavioral techniques in humans, which is an inherently subjective approach. Furthermore, behavioral noise measurements represent the combined contributions from all sites within the visual pathway, such that retinal noise cannot be separated from cortical noise. The goal of this thesis is to develop objective, electrophysiological methodologies to estimate noise that arises from different sites within the visual pathway. This was accomplished by completing the following three Specific Aims: Aim 1 developed novel noise-based electrophysiological measures. Aim 2 determined how stimulus temporal frequency affects internal noise measurements. Aim 3 developed a simplified protocol that can be applied to patient populations to study how pathology affects internal noise. **Methods.** Five control subjects and two subjects with diabetic retinopathy were recruited. Amplitude of the flicker electroretinogram (fERG; a measure of photoreceptor and bipolar cell function), the pattern electroretinogram (pERG; a measure of retinal ganglion cell function), and the flicker visual evoked potential (fVEP; a measure of cortical function) were measured as a function of stimulus contrast. Amplitude of the fundamental and second harmonic responses were derived by Fourier transforms. Measurements were performed in the absence of luminance noise and in white luminance noise of different power. Threshold, defined as the minimum stimulus contrast needed to elicit a measurable response, was derived from the amplitude measures based on Naka-Rushton fits

to the response amplitude vs signal contrast data. Threshold was then plotted as a function of luminance noise power and the data were fit with the Linear Amplifier Model, a common model of visual performance in noise. A clinically optimized protocol was developed and implemented based on the data of Aims 1 and 2. **Results.** Luminance noise had no effect on the fundamental component of the fERG and fVEP. Consequently, fundamental fERG and fVEP contrast thresholds were independent of noise power. However, noise did reduce the second harmonic component of the fERG, fVEP, and pERG. The mean internal noise (Neq) for the fERG (0.380.04) was greater than that of the fVEP (0.170.03), but similar to that of the pERG (0.330.05). Stimulus temporal frequency had no effect on Neq for the fERG and slightly increased Neq with increasing temporal frequency for the fVEP. Contrast threshold and Neq in diabetic retinopathy were normal for the fERG and fVEP, but elevated for the pERG. **Conclusion.** This thesis provides the first objective assessment of internal noise in the human visual pathway using electrophysiology. The effect of noise on the second harmonic, but not the fundamental, for each measure can be accounted for by a linear-nonlinear-linear cascade model. The surprising finding that cortical noise is lower than retinal noise can likely be attributed to cortical spatiotemporal summation, which could reduce noise in the fVEP. Finally, data from a small sample of diabetic subject provides proof-of-concept that the approach developed in this thesis has potential clinical utility.

CHAPTER 1

INTRODUCTION

1.1 Problem Statement

Noise limits all forms of communication, including vision. Understanding how noise limits human visual performance has been studied extensively for several decades using psychophysical detection and discrimination tasks. A fundamental limitation of these previous studies is that they are inherently subjective. These previous psychophysical studies are also limited in that they assess the combined effects of noise throughout the entire visual pathway.(1)

1.2 Objective

The present thesis will develop objective approaches to measure noise within the human visual system. Rather than using subjective psychophysical techniques, I will measure visual noise using electrophysiological signals, including the electroretinogram (ERG) and the visual evoked potential (VEP). This has the advantage of providing objective responses, as opposed to subjective behavioral responses. Following the development of these new techniques, a clinically-applicable paradigm was developed and implemented to collect a small amount of proof-of-concept data from a patient population.

1.3 Overview of Specific Aims

1.3.1 Specific Aim 1: Develop approaches to measure noise that arises at different sites throughout the visual pathway

The flicker ERG will be used to measure noise arising at the photoreceptors/bipolar cells, the pattern ERG will be used to measure the combined effects of outer and inner retina noise, and the flicker VEP will be used to measure noise at the primary visual cortex.

1.3.2 Specific Aim 2: Determine how stimulus temporal frequency affects internal noise measures

The methods developed in Aim 1 will be based on a single stimulus temporal frequency known to elicit robust fERG, pERG, fVEP responses. However, changes in stimulus temporal frequency might affect the fERG, pERG, fVEP responses. Hence, in Aim 2, the effects of stimulus temporal frequency on internal noise will be measured to determine if internal noise estimates change with stimulus temporal frequency.

1.3.3 Specific Aim 3: Develop a simplified protocol that can be applied to patient populations

Although the paradigms developed in Aim 1 are promising for laboratory use, the amount of time required to complete them does not make them practical for clinical use. In Aim 3 we will develop a protocol that is feasible to execute in patient populations. This simplified protocol will then be applied to visually-normal control subjects and subjects with diabetic retinopathy to obtain proof of concept data.

CHAPTER 2

BACKGROUND

2.1 Electroretinography and Visual Evoked Potentials

Non-invasive electrophysiological assessment of the visual pathway includes a wide variety of basic and advanced techniques. Of these, the two most common measures are the electroretinogram (ERG) and the visual evoked potential (VEP). These approaches have gained popularity due to their significant role in the diagnosis and monitoring of visual dysfunctions. Within the category of ERG and VEP, there are several types of tests and approaches to obtain electrophysiological data. The present study will focus on ERGs elicited by flicker and pattern stimuli (fERG and pERG) and the VEP elicited by flicker stimuli (fVEP). We have elected to focus on these responses because the fERG provides a measure of the response of photoreceptors and bipolar cells, the pERG measures the ganglion cell response and the fVEP measures the response from the visual cortex.

2.1.1 Flicker ERG

Flicker ERG (fERG) involves stimulation using uniform flashes of light. The flicker ERG is a measure of photoreceptors and bipolar cells (2). In the present thesis, it is measured using a corneal electrode that is referenced to ear. The International Society for Clinical Electrophysiology of Vision (ISCEV) publishes standards for flicker ERG stimuli and recording

conditions (3). They suggest a flicker rate of 30 Hz, but the flicker ERG can be measured at other temporal frequencies (4; 5)

2.1.2 Pattern ERG

The pattern electroretinogram (pERG) involves stimulation with temporally modulated patterned stimuli (gratings or checkerboards are most common). Importantly, there is no change in mean luminance during the temporal modulation of the stimulus, which distinguishes this stimulus from the flicker ERG discussed above. In the present thesis, it is measured using a corneal electrode, referenced to the ear. The International Society for Clinical Electrophysiology of Vision (ISCEV) publishes standards for the pERG (6). The pERG is generated largely by retinal ganglion cells. (6; 2)

2.1.3 Flicker VEP

The flicker VEP (fVEP) is elicited using the same stimulus as fERG. It is measured at the occipital scalp over the visual cortex OZ., and typically referenced to ear. ISCEV does not publish standards for fVEP. However, guidelines for measuring steady state visual evoked potentials as Electroencephalography (EEG) responses are available (7; 8). The flicker VEP is a measure of cortical function.

2.2 Noise in the Visual Pathway

Noise is present in all types of communication systems. Noise degrades the system's efficiency by adding unwanted and random variance. Noise has been studied extensively in electronics and control systems. In electronics, noise in a resistor can be modeled as a voltage or a current source representing the noise of the non-ideal (real) resistor with an ideal noise-free

resistor. It has been known that noise is present in the visual pathway as well. The same type of analysis and modeling as that used in engineering fields can be applied to the visual system, where the input is a visual stimulus and the output is the behavioral (or electrophysiological) response. In fact, the most common approach to studying noise in the human visual system was adopted from that used to calibrate audio amplifiers and radio receivers. Specifically, a known quantity of noise is added to a system and the effect of noise on the performance of the system is recorded. The amount of noise added is systematically increased until the noise added overtakes the noise naturally present in the system and causes a decrease in the signal to noise ratio (SNR) of the system. This approach has been used extensively to assess noise within the human visual system.

Historically the equivalent noise of the visual system has been described as a "dark light," which adds to the stimulus to produce the effective stimulus. Hecht (1945)(9) speculated that the effect of a single photon absorption would be indistinguishable from spontaneous (thermal) activation of rhodopsin (i.e. noise). Denton and Pirenne (1954) (10) computed, an upper bound on the number of such spontaneous activations, which they assumed was the sole source of the intrinsic visual noise. However, Barlow (1956)(11) suggested the possibility of other retinal noise sources and proposed that vision is limited by the noise in the visual pathway. It is well established that the ganglion cell responses are noisy, showing maintained discharges that are nearly independent of mean luminance (12). Cortical or neuronal noise originating beyond the retina has been researched for decades. For example, variability of spike discharge in response to identical stimuli has been credited to variances in membrane potential(13).

More recently, Pelli (1981)(14) developed a technique for characterizing the observers intrinsic noise using the Equivalent Intrinsic Noise Method. He defined random fluctuation in luminance, over time or space or both, as luminance noise. Threshold with and without a white luminance noise background added to the stimulus was measured. Threshold was found to be independent of added noise at low levels, as internal noise is larger than the external noise for low levels of external noise. However, at higher external noise levels, threshold increases in proportion to the added noise. I shall implement the same technique to estimate intrinsic noise.

CHAPTER 3

METHODS

3.1 Stimulation

For stimulation, I have used the Espion Visual Electrophysiology System from Diagnosys, LLC, which has functionalities for both ERG and VEP. Stimuli were generated using the Espion on a CRT display (NEC monitor; FE2111SB). The CRT has a resolution of 1280 x 1024 pixels with a 75 Hz refresh rate. The parameters used are described below.

3.1.1 Flicker

The stimulus for flicker ERG and VEP is a series of uniform luminance flashes. The mean luminance of all stimuli was 32 $\mathtt{cd}/\mathtt{m}^2$. The stimulus contrast ranged from 0% to 40% (Michelson contrast) in steps of 0.15 log units. The stimulus temporal frequency was 6.25 Hz (160 millisecond period).

3.1.2 Pattern

The stimulus for pattern ERG is a black and white reversing square-wave grating. The spatial frequency was 1 cycle/degree with each bar subtending 0.5 deg. The stimulus is reversed at 6.25 Hz (12.5 reversals per second). The mean luminance if the screen was constant at 32 $\mathtt{cd}/\mathtt{m}^2$. Consistent with the flicker stimuli, the contrast of the pattern stimuli ranged from 0% to 40% (Michelson contrast) in 0.15 log unit steps.

3.1.3 Noise

The noise was created using a Macintosh G4 computer and scripted in a programming tool MATLAB (MathWorks, Natick, MA), using Psychophysics Toolbox (Brainard D. 1997). The noise was displayed on the same type of CRT used for the stimulus display (NEC; FE2111SB). The white noise consisted of a uniform field whose luminance was modulated randomly in time, above and below the mean. The field was changed at a temporal frequency of 25 Hz. Noise power is defined as the ratio of mean luminance and standard deviation of luminance from the mean (14; 15) given in Equation 3.1

$$E = c_{RMS} A T \tag{3.1}$$

where E is contrast energy, c_{RMS} is RMS contrast, A is area, T is duration. The noise RMS contrast in the present study ranged from 0.0 to 0.28 RMS in 0.3 log unit steps.

The image from the CRT that displayed the noise was combined with the image from the CRT that displayed the stimulus using a half-silvered (teleprompter) mirror. As there is no need to temporally synchronize the noise and stimulus displays, the noise was set to be free running during the experiments. Luminance values for both the stimuli and noise in the experiments were calibrated with a linearized look-up table. Luminance was measured using a Minolta LS100 luminance meter. The displays were measured with a photocell and an oscilloscope for verifying the the temporal features. As an additional check for potential luminance artifacts

in the pERG stimuli, a semitransparent paper was placed in between the monitor and the eye and no visible flicker was observed.

3.2 Recording Equipment

3.2.1 Electrodes

The ERG signal was recorded using a DTL Plus corneal electrode. The DTL Plus is a single use, ultra-low impedance, medical grade silver/nylon electrode. The cornea is covered when required with ionic conductive eye drops called Refresh plus lubricant as recommended by ISCEV. Standard skin silver-silver chloride cup electrodes are used for recording visual evoked potentials.The skin in cleaned for better connection. Elefix (Nihon Kohden Inc) paste for EEG is also used for better and a stable electrical connection to the skin of the scalp. The scalp electrodes are placed according to the International 10/20 system. The VEP electrode is placed on the occipital scalp(O_Z) over the visual cortex. The reference electrodes for both ERG and VEP are placed on the earlobe. This arrangement permitted high SNR. A separate electrode is attached at F_PZ (forehead) and connected to the ground. Without any stimuli, the base response for ERG is stable.

3.2.2 Recording System

The Diagnosys E3 system was also used to record the electrophysiological responses. It contains a 32-bit DAC amplifier with high common mode rejection of <100dB and recording are obtained through 2DC channels. I have used the sampling frequency of 2 kHz, which is double the ISCEV standard recommendation of 1 kHz. There is a high pass filter with cutoff of 100 Hz and low pass filter with cutoff of 1 Hz. There is an artifact rejection of blink, and eye

movements, where the thresholds are set at ±100 μV. There is also a rejection of high-frequency noise and a notch/screen filter for 60 Hz to avoid the AC mains line noise peak.

We recorded 50 sweeps with a sweep of duration 1280 milliseconds. Thus, each sweep contains 8 cycles. The 50 sweeps are averaged to reduce variability and background noise. The recording system provides a real-time display of the recorded signal.

The subject is seated comfortably with a chin-rest. Monocular stimulation is used, as recommended by ISCEV for VEP (16). A red cross mark is placed at the center of the screen so that the subject can focus to ensure a good quality signal. The subject is asked to fixate the red cross and to minimize eye blinks. Rest breaks are given as needed.

3.3 Analysis

All analyses were performed with custom-written MATLAB software. Data analysis follows a series of steps, beginning with artifact removal and ending with spectral analysis as follows:

First, blink and eye movement artifacts that result in large amplitude fluctuations in some sweeps, are removed. This was accomplished by omitting individual sweeps that exceeded 80 microvolts, as a response this large is not expected under the stimulus conditions used. Secondly, VEPs can be contaminated by the alpha response (steady-state brain potentials) in the frequency band of approximately 8-12 Hz. An alpha response, when averaged with the stimulus-driven VEP, might introduce an artifact. The alpha response, although periodic, is not time-locked to the stimulus, which makes this artifact apparent. To omit the alpha-contaminated waveforms, we reject sweeps with an amplitude of higher than 10 microvolts at frequencies near 10 Hz, as amplitudes exceeding 10 microvolts are not expected to be elicited by

our stimuli. This approach successfully suppressed alpha-based noise. The third issue that was addressed in MATLAB is signal drift (trend artifact). The trend artifact is a slow increase or decrease in the response that results in low-frequency amplitude in the frequency spectrum. To minimize the trend artifact, we first average the signal and then detrend it by using polynomial wave fitting functions in MATLAB, using the least squares method.

The detrended average waveform is then processed with the Fast Fourier transform(17) to calculate response amplitude and noise. A key component of the response occurs at the stimulus frequency (the fundamental). In addition, there are typically large amplitude components at multiples of the signal frequency (the harmonics). In the present thesis, we calculate the signal amplitude at the fundamental and second harmonic frequencies. In addition, noise is estimated as the average for the two neighboring spectral frequencies(18).

3.4 Noise Model

To measure the noise within the visual pathway, we must first obtain measures of the threshold ERG and VEP responses. The threshold is defined as the minimum stimulus contrast needed to elicit a response of a criterion amplitude.

First, we measure the response amplitude (fundamental or second harmonic) in the absence of noise at a series of different stimulus contrasts. Amplitude is calculated as a function of signal contrast and the threshold measurements are calculated with a Naka-Rushton function, defined in Equation 3.2.

$$R(C) = R_{max}\frac{C^n}{C^n + K^n} \tag{3.2}$$

Where R is the response to contrast C, R_{max} is the maximum asymptotic response amplitude; n is proportional to the slope of the curve, and K is the contrast required to produce half the max amplitude. K and n are fitted to limit the error between the measurements and the Naka-fit. R_{max} is kept constant at 1 since the data are normalized to the maximum response amplitude. The K parameter was used to define the threshold (i.e. the stimulus contrast needed to elicit half of the maximum response). This procedure was repeated for measurements made at different levels of white luminance noise with example results shown in Figure 1.

The blue trace in Figure 1A is the signal and the red trace is the noise that is added to signal as seen the black trace. The noise power is varied and fit using Equation 3.2. In Figure 1B the blue curve in is the signal with no noise and red is maximum noise with ascending order of noise power. The threshold is calculated as the contrast required to produce half of maximum amplitude.

Internal noise is estimated by implementing the "Equivalent Input Noise" technique. The results are typically evaluated using the "Linear Amplifier Model" (LAM). This model was originally devised to model human psychophysical thresholds in noise, but we have adapted this model to permit analysis of electrophysiological signals in noise. For each noise level, the contrast threshold is calculated as a function of noise power (Figure 1C) and the data are fit with the LAM(19)

$$\log C_t = 0.5[\log(K) + \log(N + N_{eq})] \tag{3.3}$$

where N is the external noise added, N_{eq} is the "equivalent intrinsic noise" (an estimate of noise in the visual pathway and K is an estimate of efficiency)(14). Figure 1C uses data

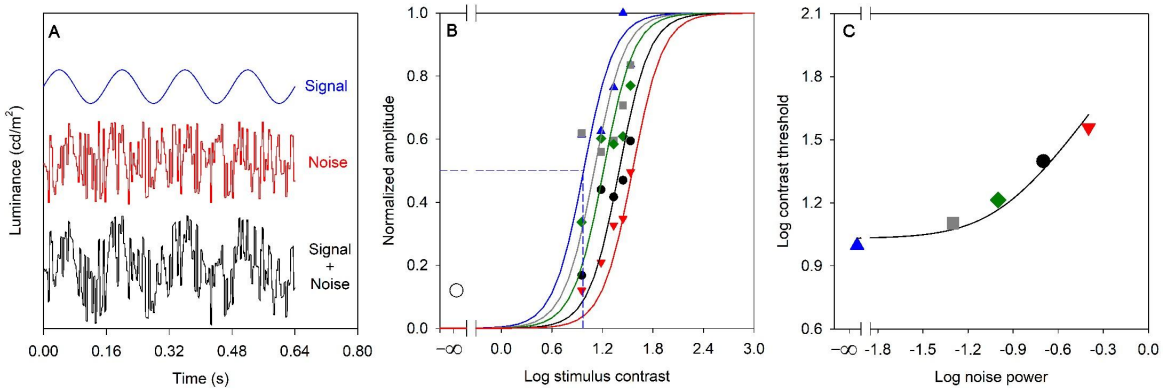

Figure 1. A Description of Methodology

obtained from Figure 1B to plot log contrast threshold as a function of noise power. N_{eq} is thus obtained from Equation 3.3.

CHAPTER 4

RESULTS

4.1 Specific Aim 1

Develop approaches to measure noise that arises at different sites throughout the visual pathway

Using the approach described in Fig. 1, contrast threshold was derived for each measure (fERG, pERG, and fVEP). I recruited four control subjects. Figures 2 to 4 shows the graph of log contrast threshold as a function of log noise power. In these figures, each subject is represented by a different symbol.

Data for the 6.25 Hz fERG are shown in Figure 2. Figure 2a shows that noise had no effect on the fundamental flicker ERG threshold. These data are fit with linear regression lines with a slope of 0 because the threshold is independent of noise. However, there is a noticeable effect of noise on the second harmonic of the fERG, as shown in Figure 2b. As noise power was increased, the stimulus contrast needed to produce a measurable fERG 2^{nd} harmonic (i.e. threshold) increased. A 1 log unit increase in noise increased threshold by 0.11 log units. Note that there were differences in threshold (approximately 0.3 log units) among the four subjects for results measured with the no noise condition (leftmost data points) and that the thresholds converged for three of the four subjects in the maximum level of luminance noise used in this thesis. As discussed below, the differences among these three subjects can be attributed to

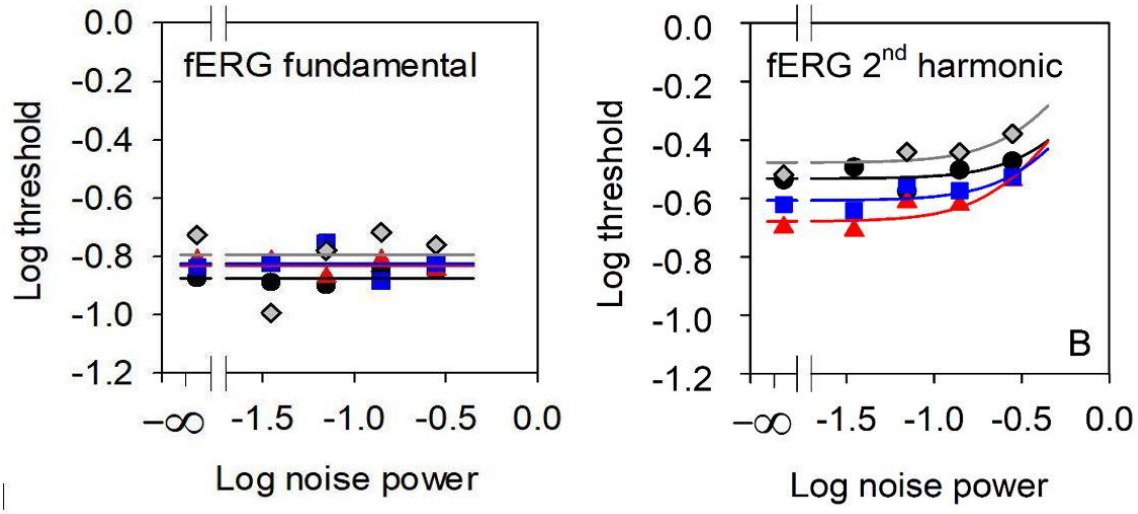

Figure 2. Effect of noise on contrast threshold for fERG

different levels of internal noise. The thresholds for subject 4 (gray) were generally shifted upward for all noise powers, indicating lower efficiency than the other three subjects.

Figure 3a depicts the fVEP fundamental and figure 3(b) second harmonic (3b) elicited by 6.25 Hz flicker. Here, there is no effect of noise on the fVEP fundamental. These data are fit with linear regression lines with a slope of 0. Although the same general pattern was observed for each subject, the results for each subject are displaced vertically. Figure 2B shows a larger

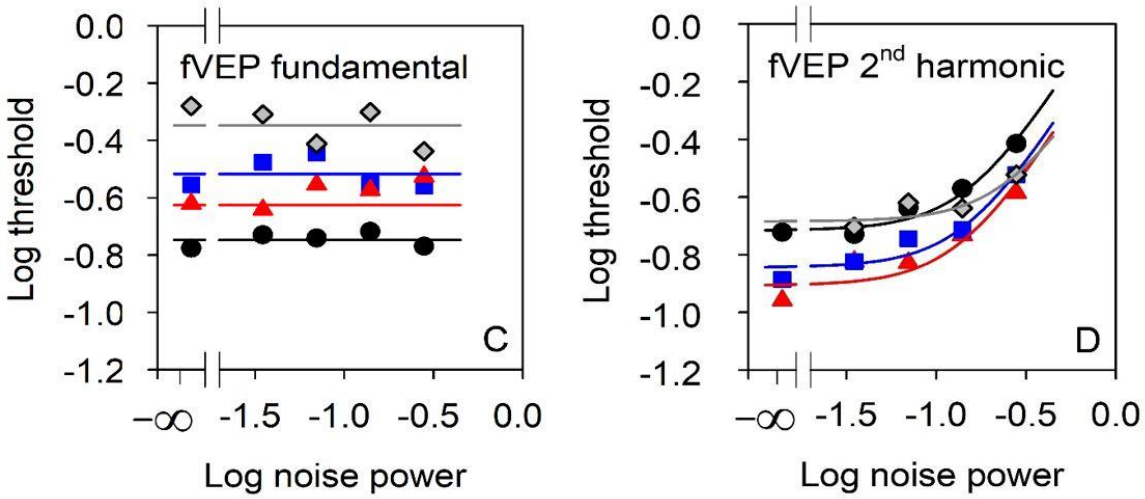

Figure 3. Effect of noise on contrast threshold for fVEP

effect of noise on the second harmonic of the fVEP, as compared to the fundamental fVEP or the fERG second harmonic. A 1 log unit increase in noise increased threshold by 0.32 log units.

Figure 4 plots log contrast threshold for the pERG vs log noise power for each subject. As noise power increased, there was an increase in the log threshold pERG. A 1 log unit increase in noise increased contrast threshold by 0.18 log units. Note that there are differences in

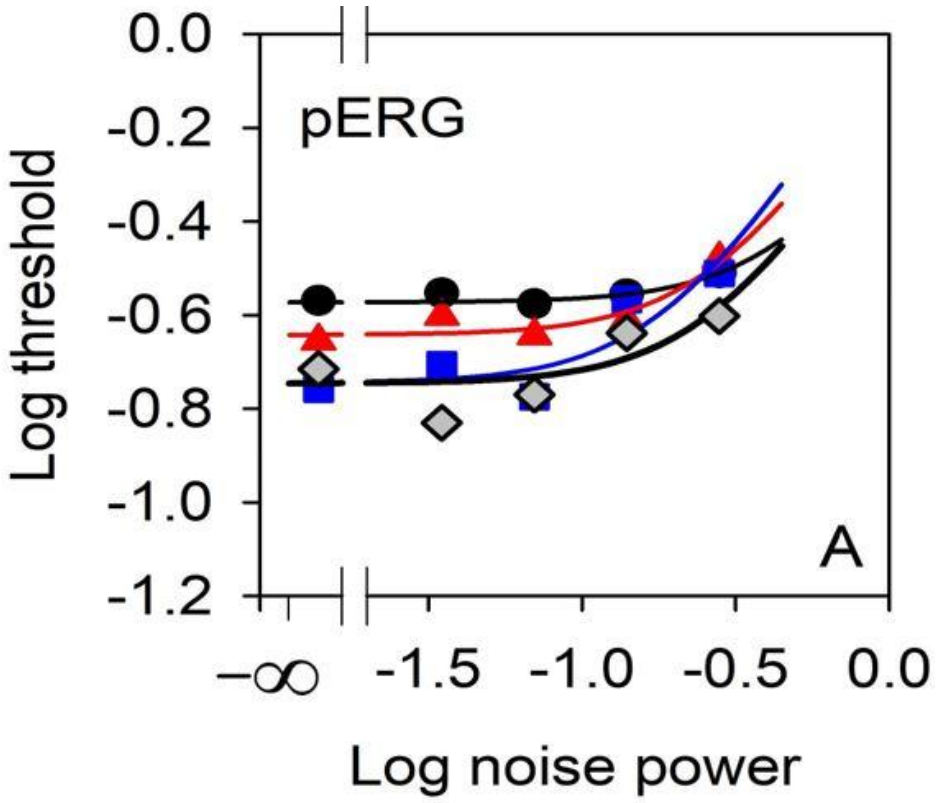

Figure 4. Effect of noise on contrast threshold for pERG

contrast threshold among the four subjects in the absence of noise (approximately 0.2 log unit differences), but the functions for the subjects tend to converge in high levels of noise.

The intrinsic noise (N_{eq}; the knee-point of the curves in Figures 2, 3, 4) present in each measure was derived by Equation 3.3 for each subject. Figure 5 shows the mean N_{eq} (±SEM) for the four subjects for each measure

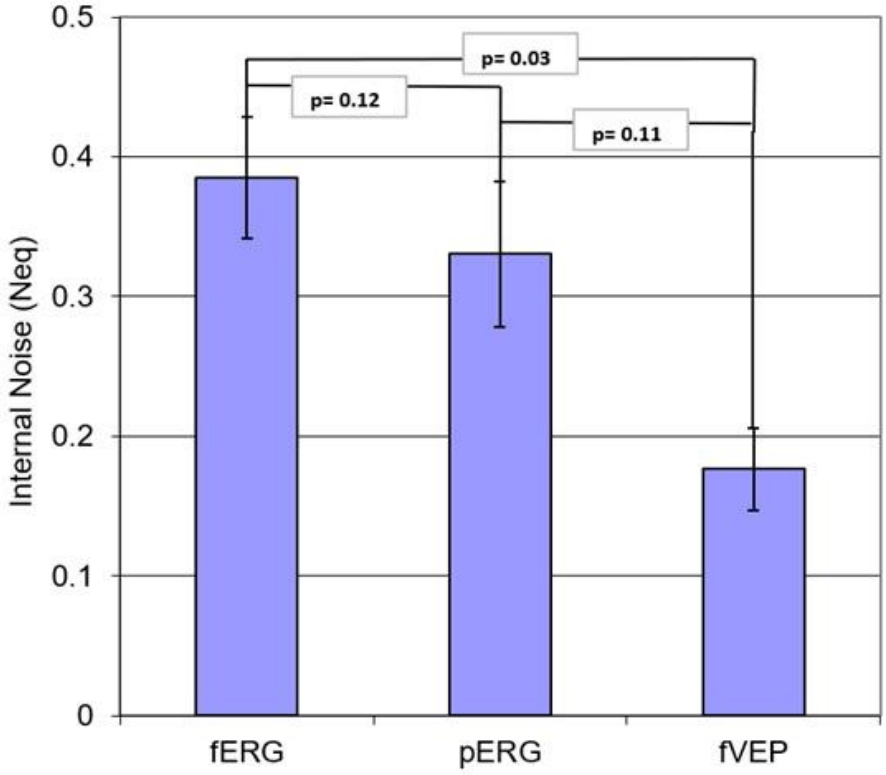

Figure 5. Comparison of mean N_{eq} for each measure

The mean internal noise (N_{eq}) for the fERG (0.38±0.04) was larger than that of the pERG (0.33±0.05) and fVEP (0.17±0.03). Paired t-tests indicated that N_{eq} for fERG was significantly greater than that of fVEP (p=0.03). However, the fERG N_{eq} was not significantly greater than that of the pERG N_{eq} (p = 0.12). Likewise, the pERG and fVEP N_{eq} values were not statistically different, but there was a non-significant trend (p = 0.11). Note that the sample

size was small (N=4) and with a larger sample size, a significant difference may become apparent between the pERG and fVEP N_{eq} values.

4.2 Specific Aim 2

Determine how stimulus temporal frequency affects internal noise measurements

The data obtained in Aim 1 were restricted to a temporal frequency of 6.25 Hz. Consequently, it is unknown whether similar results would be obtained at higher or lower stimulus temporal frequencies. It is possible that changing temporal frequency will have different results on the fERG and fVEP measures. Specifically, based on psychophysical measurements, Pelli (1990) (20), Raghavan (1995) (21) and Silvestre, Arlo, Allard (2018) (22) found that noise that is assumed to arise at the retina is independent of temporal frequency, whereas noise that was assumed to arise at the cortex scales with temporal frequency. If this is correct, then we predict that from the fERG N_{eq} will be independent of temporal frequency, as this response arises from the retina, whereas the fVEP N_{eq} should increase as temporal frequency increases, as this response arises from the cortex. To test this prediction, we applied the methods developed in Aim 1 at various stimulus temporal frequencies.

Figure 6 plots the response amplitude of the fERG and fVEP second harmonics for three different stimulus temporal frequencies: 5 Hz, 6.25 Hz, and 10 Hz. For clarity, only three low contrast levels (5%, 10%, and 14%) are shown. The black data points represent the response measured for the no noise condition and red is the response for the highest noise condition (0.28 contrast RMS). Each data point represents the mean (±SEM) of three control subjects. In general, the addition of luminance noise produced a small reduction in the amplitude of the

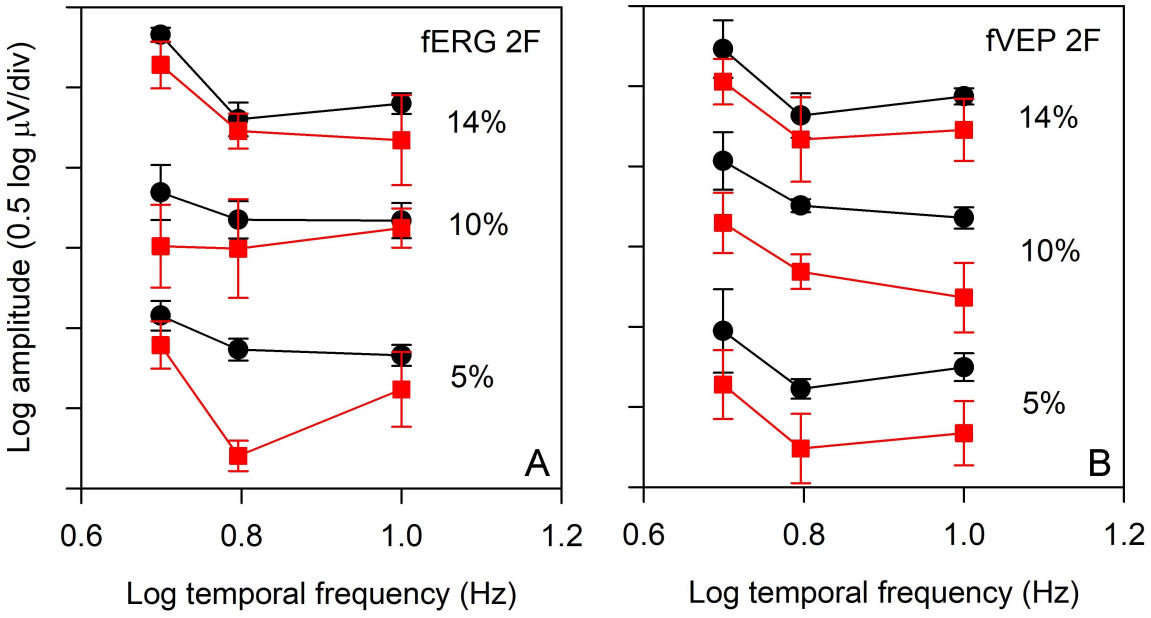

Figure 6. Amplitude of signal response for 3 temporal frequency in absence and presence of external noise

2nd harmonic of the fERG at each temporal frequency, consistent with Figure 2. Figure 6B shows that the effect of adding noise on the fVEP amplitude is larger than that observed for the 2nd harmonic of the fERG. In general, noise appears to have similar effects on the amplitude for each stimulus temporal frequency.

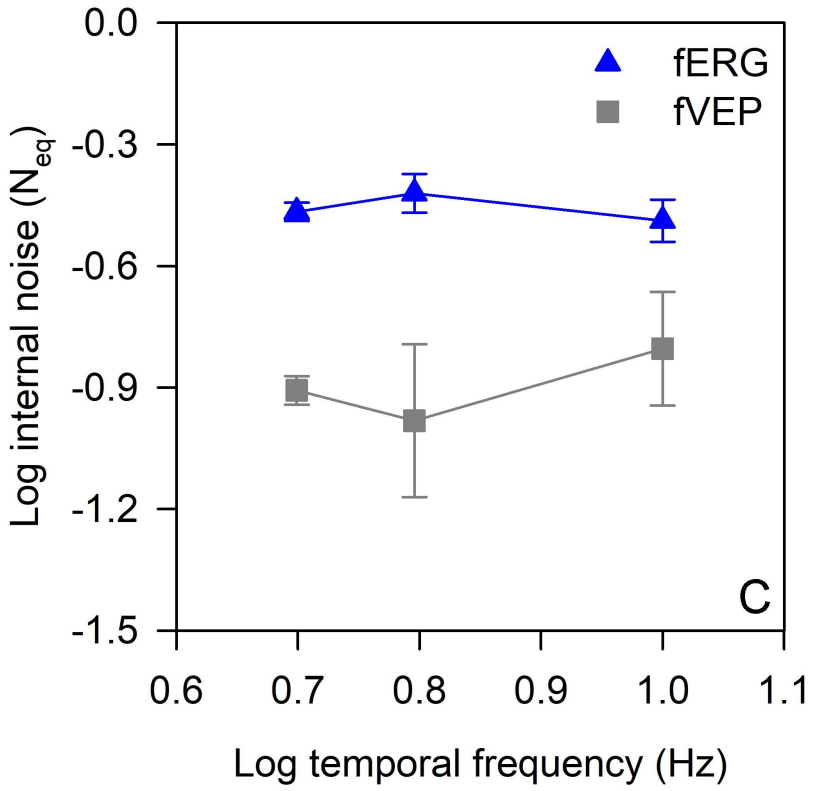

Figure 7. Effect of temporal frequency on internal noiseN_{eq}

We estimated equivalent intrinsic noise (N_{eq}) using Equation 3.2, as described above for each temporal frequency. A graph of internal noise as a function of stimulus temporal frequency is shown in Figure 7. There is no effect of frequency on the fERG. This is consistent with the characteristics of retinal noise predicted in psychophysical studies by Pelli (1981) (14) and Raghavan (1995) (21). For the fVEP, there was a slight increase in N_{eq} (0.1 log units) as temporal frequency increased from 6.25 to 10 Hz. This trend is in the expected direction of that

predicted by Pelli (1981) and Raghavan (1995). If measured at higher temporal frequencies, the N_{eq} measured for the fVEP may increase further, but the fVEP response becomes very small at higher temporal frequencies, which makes measurements difficult. Interestingly, internal noise for the fERG was approximately 0.3 log units higher than internal noise for the fVEP. As discussed below, this argues against a simple addition of noise throughout the visual pathway.

4.3 Specific Aim 3

Develop a simplified protocol that can be applied to patient populations

The protocols developed and implemented in Aims 1 and 2 proved useful to collect data for various stimulus contrasts and temporal frequencies in different levels of luminance noise. However, each session duration was approximately 75 minutes and the flicker and pattern stimulus data could not be collected in the same session. This is suitable, although not ideal, in a laboratory setting but not possible in a clinical setting.

Hence, we developed a clinically-applicable protocol that required approximately 27 minutes to complete, including both flicker and pattern stimulus conditions. This is a reasonable amount of time for application in patient populations. Table 1 shows select parameters of the full paradigm developed in Aim 1 and the simplified paradigm which is applied clinically. To expedite data collection, we reduced the number of noise conditions from five to two (using only the no noise and 0.28 c_{RMS} conditions). The recording duration of a single sweep was halved from 1280 milliseconds to 640 milliseconds (this also reduced the number of cycles per sweep from 8 to 4). The number of stimulus contrasts was reduced from 7 to 4 for both flicker and pattern stimulus with an additional 0% stimulus contrast. Despite the abbreviated

paradigm, we obtained responses similar to the responses in Aim 1. We implemented this protocol in 3 control subjects and 1 subject with diabetic retinopathy.

TABLE I

COMPARISON OF PARAMETERS USED IN AIM 1 AND AIM 3

	Number of Stimulation Contrasts	Number of Noise Conditions	Time(minutes)
Full Paradigm	8+7=15	5	75
Clinical Paradigm	5+4=9	2	27

Figure 8 presents the log contrast threshold as a function of log noise power for these three control subjects and one diabetic patient. The curves are generated using the methodology developed in Aim 1. The gray region which represents the normal range of three control subjects was inferred by first considering the maximum and minimum values of contrast threshold for each condition of noise power. The maximum and minimum values were then fit into Equation 3.2 to define the normal range as shown in Figure 8. The diabetic patient is represented by the blue data points. We observe that contrast threshold for the subject with diabetic retinopathy is in (or near) the normal range of that of the control subjects for fERG . However, the contrast threshold for subjects with diabetic retinopathy is greater than that of control subjects for pERG. Likewise, the patient with diabetic retinopathy had normal N_{eq} for the

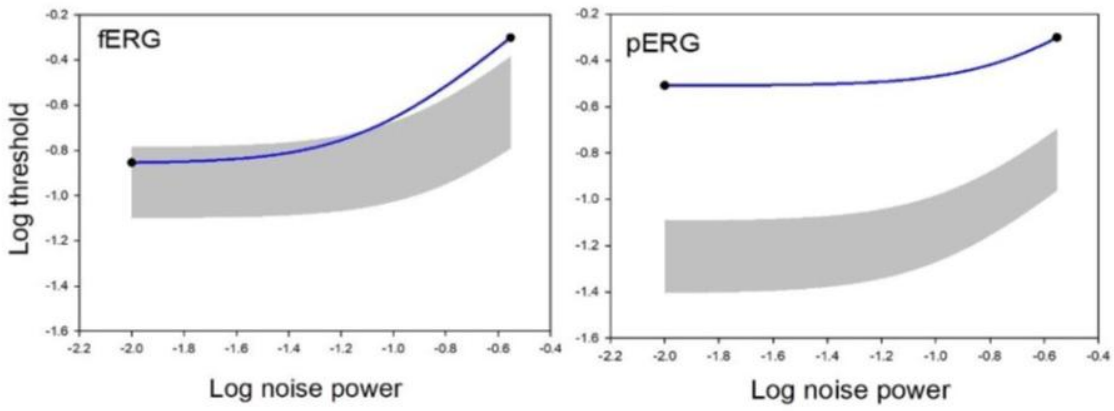

Figure 8. Effect of noise power on contrast threshold in diabetic retinopathy; Gray area is the normal range of control subjects defined in the text

fERG but elevated N_{eq} for the pERG. The data obtained for fVEP was extremely noisy with no measurable signal. Hence, fVEP was excluded.

CHAPTER 5

DISCUSSION

The aim of this thesis was to develop electrophysiological methods to measure and study internal noise of the human visual pathway. Using fERG, pERG, and fVEP, internal noise arising at the photoreceptors/bipolar cells, ganglion cells and visual cortex was measured respectively.

5.1 Specific Aim 1

The goal of Aim 1 was to develop approaches to measure noise that arises at different sites throughout the visual pathway. The results showed that noise had no effect on the fundamental fERG and fVEP, but an increase in contrast threshold was observed for the 2^{nd} harmonic components of these responses. This finding can be explained by an existing sandwich model of processing proposed by Spekreijse (1966). This model suggests a linear-nonlinear-linear cascade of filters present in both the retina and primary visual cortex that generates the ERG and VEP.

Figure 9 explains the working of the sandwich model for a 6.25 Hz stimulus. This figure assumes an ideal, noise free response generated by the photoreceptors.

The gray data trace is the response of the photoreceptors at 6.25 Hz. The peak to trough amplitude is set to be 200 microvolts in this example. In contrast to the linear photoreceptor response, nonlinear behavior arises at the bipolar cell level, as seen by the green trace. Thus, at this second level there is full-wave rectification of the bipolar cell response, which introduces the second harmonics. The amplitude of the bipolar response is 100 microvolts in this example,

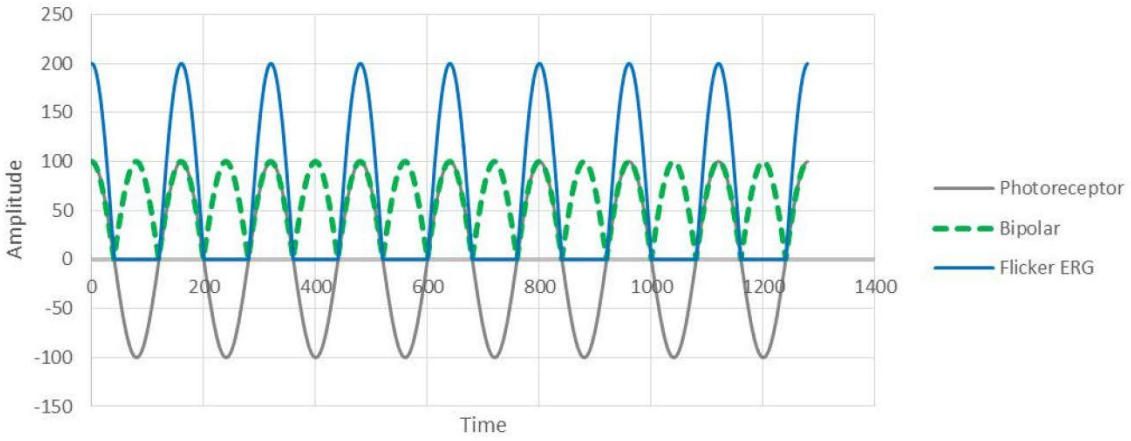

Figure 9. Responses of an ideal visual system using Sandwich model

due to the rectification. The flicker ERG is the sum of the photoreceptor and bipolar responses (blue trace). The Fourier transforms of thee traces shown in Figure 9 are shown in Figure 10. The photoreceptor response is shown at the fundamental frequency (6.25 Hz). There are no harmonics present, as the photoreceptors response is assumed to be sinusoidal. In comparison, the response from bipolar has multiple harmonics, but lacks the fundamental frequency (middle

panel). The flicker ERG is obtained by adding them has the response at fundamental as well as the presence of harmonics (lower panel).

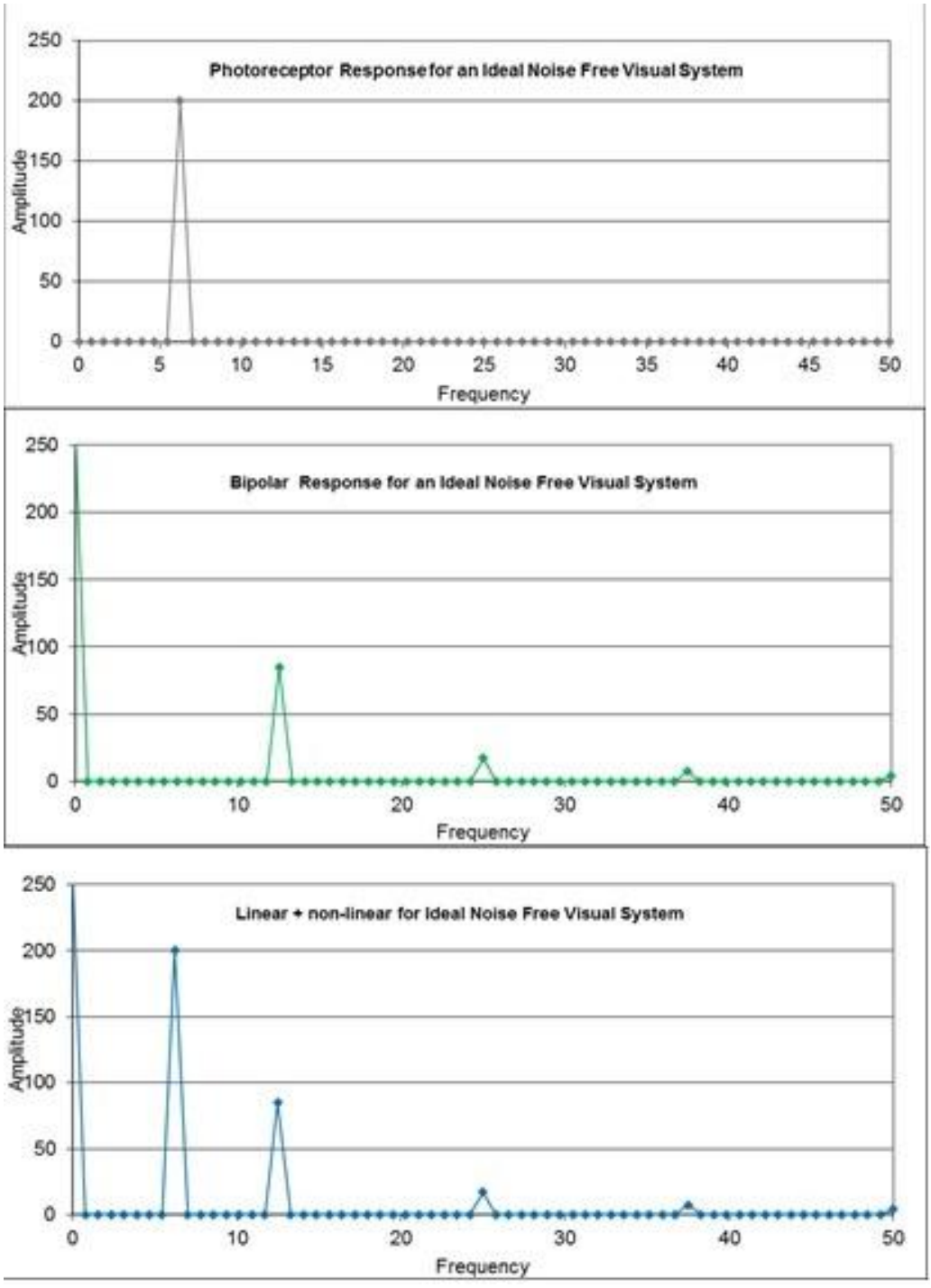

Figure 10. Responses of a noisy visual system using Sandwich model

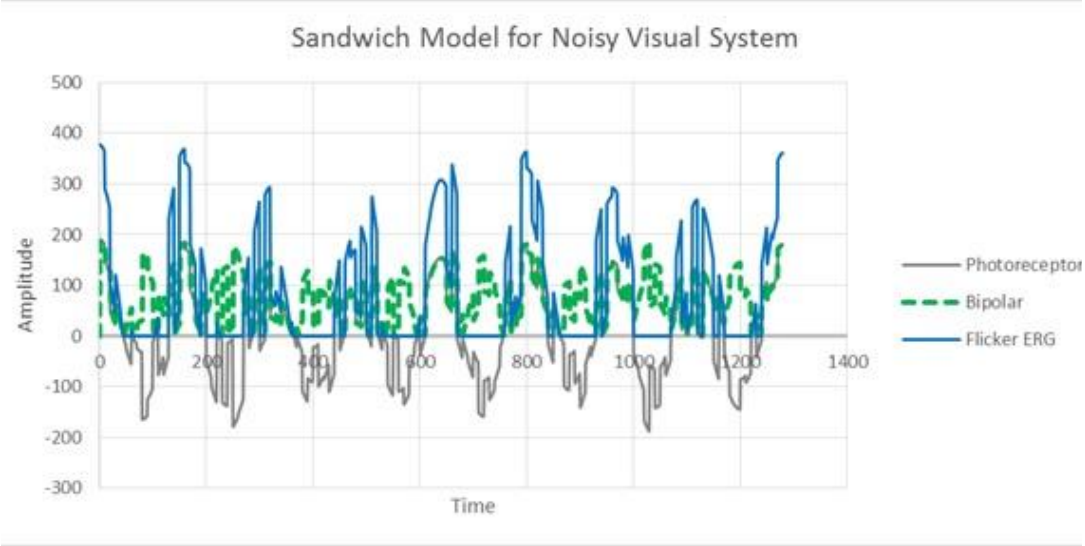

Figure 11. Responses of a noisy visual system using Sandwich model

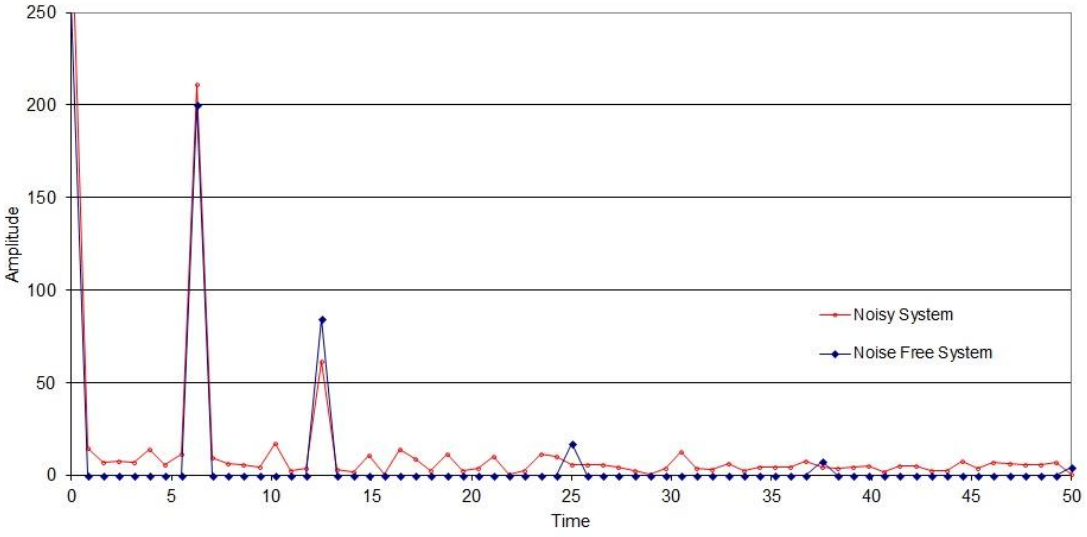

Figure 12. FFT Spectrum of an ideal and a noisy visual system using Sandwich model

Spekreijse(1966)(23) proposed that if the noise in the visual pathway occurs before the nonlinearity that generates the harmonics(at the photoreceptors), then both the signal and the noise will be rectified, which will have minimal effect on the fundamental response recorded at the electrode but will reduce the 2^{nd} harmonic component. We modeled this by adding white temporal noise to the signals shown in Figure 9 as shown in Figure 11. Thus, we can say that addition of noise is expected to reduce the harmonic amplitude without attenuating the fundamental response, as seen in Figure 12.

A reduction in stimulus amplitude in presence of external noise was observed at the 2^{nd} harmonics for all subjects as shown in Figure 6. This increases the contrast threshold required as consistent with the findings in Aim 1.

An additional interesting finding of Aim 1 is that the mean internal noise for fERG was significantly greater than that of the fVEP. This finding argues against a simple summation of noise sources throughout the visual pathway. That is, if noise summed throughout the visual pathway, then the VEP noise would be expected to be greater than the ERG noise. This finding is consistent with previous suggestion that the dominant source of noise is at the retina, but extends this suggestion to show that cortical processing may reduce the total amount of noise within the visual pathway. This can likely be attributed to cortical spatiotemporal summation, which could reduce noise in the fVEP.

5.2 Specific Aim 2

The goal of Aim 2 was to determine how stimulus temporal frequency affects internal noise measurements. The results of Aim 2 showed that N_{eq} measured for fERG did not change with

increasing frequency. This finding is consistent with the prediction of Raghavan (1995), who proposed that retinal noise is independent of all spatiotemporal frequency. In contrast, the results of Aim 2 showed that N_{eq} increased slightly with stimulus temporal frequency. This finding is consistent with the prediction Raghavan (1995) who suggested that cortical noise should increases as stimulus temporal frequency increased. Given the marked amplitude loss of the VEP at moderate to high temporal frequencies, we did not attempt to measurable the fVEP at higher frequencies. Future studies could be designed to measure N_{eq} at temporal frequencies above 10 Hz to further test the predictions of Raghavan (1995). The results of the present Aim suggest that any frequency between 5 and 10 Hz should be suitable for measurements of internal noise in the fERG, pERG, and fVEP.

5.3 Specific Aim 3

The goal of Aim 3 was to develop a simplified protocol that can be applied to patient populations. Data from a small sample of diabetic subject provides proof-of-concept that the approach developed in this thesis has potential clinical utility. The data from diabetic and control subjects was were recorded using the clinical protocol. Although only a subset of measurements were made in the clinical paradigm, the results obtained from 3 control subjects were highly consistent with the results obtained using the complete paradigms discussed in Aim 1.

For the diabetic subject, threshold and internal noise were below the upper limit of normal for the fERG, but were above the limit of normal for the pERG (i.e. abnormal). The elevated pERG internal noise is consistent with the known abnormalities in inner-retina structure and

function. Specifically, other measure of inner retina function, such as the oscillatory potentials of the flash ERG (24) and reductions in the pupil response mediated by intrinsically photosensitive retinal ganglion cells (25) have been shown to be abnormal. Likewise, thinning of the retinal ganglion cell layer has also been shown in diabetic retinopathy (26).

CHAPTER 6

CONCLUSION

Internal noise was successfully measured at different sites within the visual pathway using electrophysiological signals fERG, pERG, fVEP. Thus, internal noise is estimated for photoreceptors, RGCs, and cortex respectively. The surprising finding is that internal noise may be higher in the retina compared to visual cortex. This opens path for studies and future work for the processing capability of the visual cortex. Electrophysiological results obtained across temporal frequency are consistent with predictions based on psychophysical measures. Internal noise elevation in diabetics measured psychophysically may be associated with RGC (but not photoreceptor) processing. This thesis provides the first objective assessment of internal noise in the human visual pathway using electrophysiology.

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