PHOTOPIC RAPID ADAPTATION IN THE ELECTRORETINOGRAM:
A WHITE NOISE ANALYSIS

Thesis by

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ABSTRACT

When light enters the eye it initiates a complicated chain of events which eventually lead to the sensation of vision. The electroretinogram (ERG) is a widely used indirect measure of the visual system. One of the most interesting aspects of the visual system, adaptation, is its ability to function over a very wide range of incoming light intensities. This thesis is concerned with the study of the retina during small, fast changes in the state of adaptation.

To study rapid adaptation, a new technique was developed which may also be used for looking at other biological systems. Kernels (a form of cross-correlation), when used with white noise stimuli, have proven very useful in the study of the dynamics of photopic rapid adaptation. Using first order kernels we have probed the wide range of adaptation and compared kernels to flash responses. With the second order kernel we found evidence that a late (125 ms) wave in the ERG is caused by rapid adaptation, identified the components of highly abnormal ERG's and obtained basic information about the internal organization of a system. The third order kernel characterized suppression-recovery in the photopic ERG.

We then speculated on the correlation of our results with some of the prevailing views of the organization and operation of rapid adaptation in the photoreceptors of the retina.

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1) Systems
(What are we looking at?)

1a -- introduction

When light enters the eye it initiates a complicated chain of events which eventually lead to the sensation of vision. Both basic researchers and clinicians are interested in the details of these processes, but when human subjects are being studied there are limitations on the methods which can be used. It is not practical or ethical to do the obvious and dissect through the overlaying tissue to study the components of the visual system directly. Thus some indirect method is needed which will reflect the internal operation of the system.

The electroretinogram (ERG) is a widely used indirect measure of the processing within the visual system. It is an electrical signal recorded on the cornea when flashing a light into the eye (Figure 1A.1). It basically reflects the response of the first few layers of the retina, not individual cells, but the mass response of whole classes of cells (Armington, 1974; Rodieck, 1973; Brown, 1968).

One of the most interesting aspects of the visual system is its ability to function over a very wide range of incoming light intensities, about 10 log units (a log unit is a ratio of ten, so 10 log units is 10^{10}, or 10 billion to 1). This ability of the visual system to adjust its sensitivity is called adaptation. A superficial explanation of this phenomenon is the pupil response, the light is attenuated by making the pupil smaller. But, the pupil can only change its area by about a factor of 16, which is obviously not enough to account for the whole effect (Werblin and Dowling, 1969).

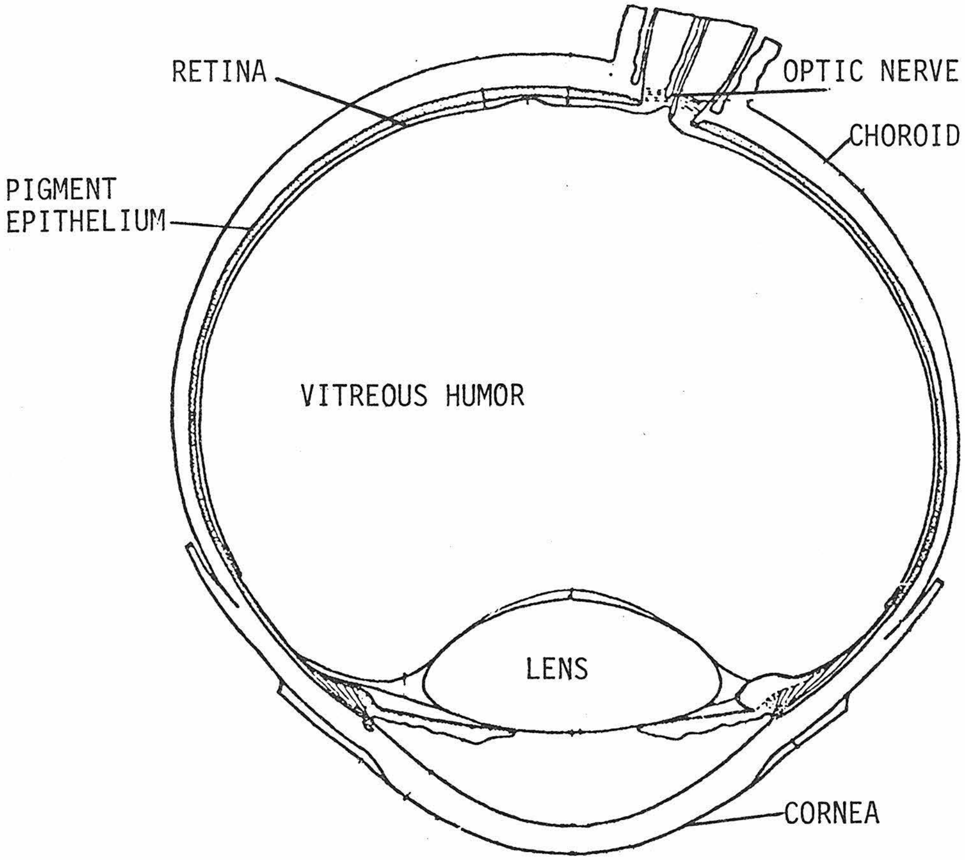


Figure 1A.1 The eye.

Adaptation might then occur in the retina, and if so it should have some effect on the ERG. When the ERG is measured at different levels of light intensity, two things happen; 1) the amplitude does not change much, compared to the large changes in light levels, and 2) its shape changes. This suggests that the ERG can be used to study some of the characteristics of the process of adaptation.

One way to study adaptation using the ERG is to collect a series of responses at different light levels and then compare them. These responses are obtained at each level after the effects of changing the levels has died out and the system has reached a steady condition. The time needed for the system to come to an equilibrium varies from minutes when the change is very large, to fractions of a second when the changes are small. Likewise, the time needed for the system to alter its adaptation is minutes when the change needed is large, to fractions of a second when the change needed is small. The large, slow changes have been studied in great detail, but the small fast ones have not.

This thesis is concerned with the study of the retina (as reflected in the ERG) during small, fast changes in the state of adaptation (figure 1A.2). A characterization of rapid adaptation will help in the overall understanding of the interesting question of adaptation in the visual system. To study rapid adaptation a new technique will be developed which may also be used for looking at other biological systems

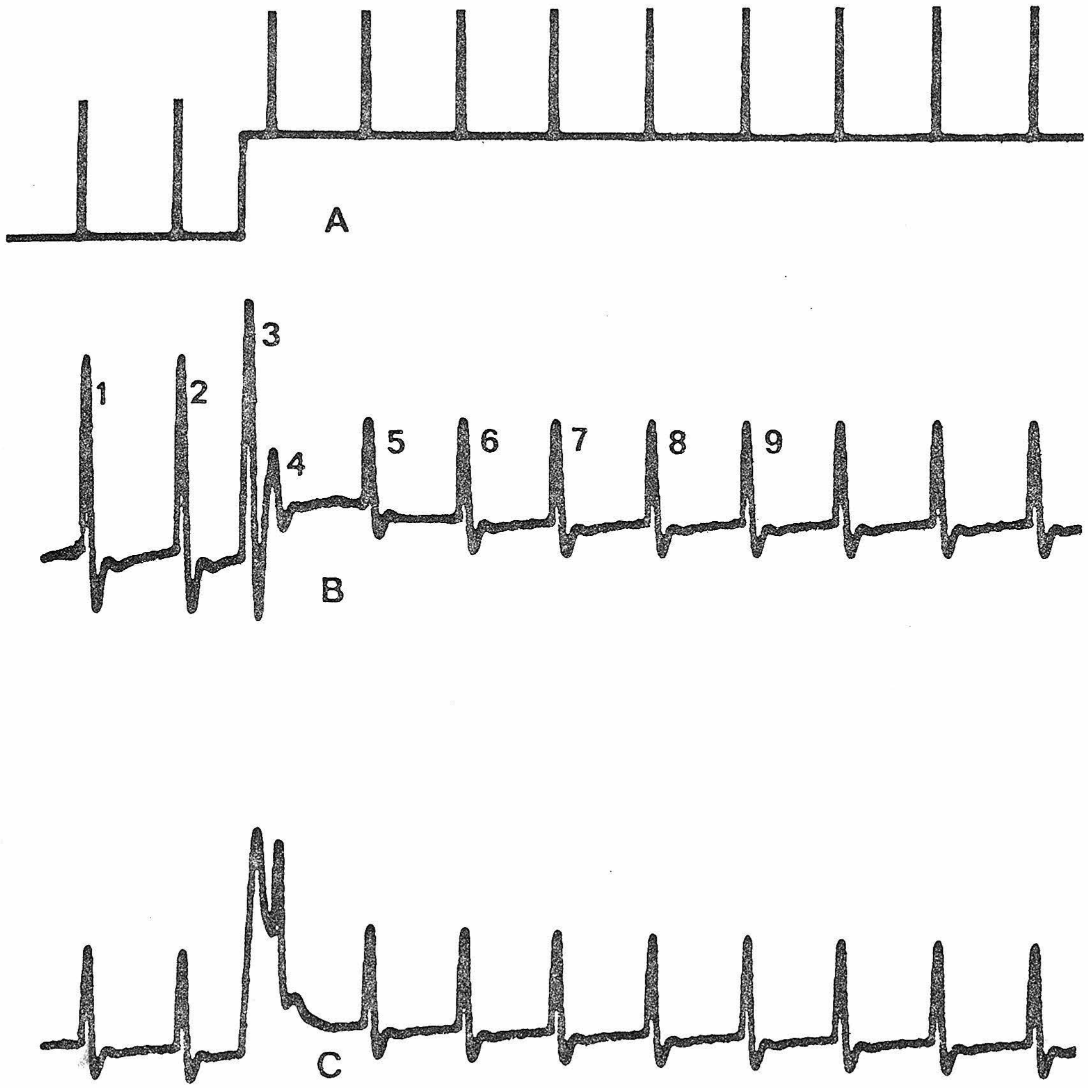


Figure 1A.2   Adaptation

This figure illustrates the difference between the responses of an adaptive system vs. a system which does not adapt.

A) The stimulus, a train of flashes superimposed upon a step change in the background level.

B) The response of an adaptive system. Notice that responses 1, 4, and 9 are not the same size.

C) The response of a non-adaptive (linear) system. Notice that all of the responses are the same size. Do not confuse the transient response to the step with adaptation.

1b -- retinal physiology

The present understanding of the physiology of the retina has been described elsewhere many times in great detail and only a brief summary will be described here (Brown, 1968; Rodieck, 1972). The measurement of the ERG of humans is usually done by placing the recording electrode on the cornea of the eye. The reference electrode may be placed almost anyplace else, although it is generally best to place it close to the eye (Armington, 1974).

If an electrode is placed within the retina, many cell classes would be seen to produce a measurable response, but when measured at the cornea two classes dominate the response, the receptors and Mueller cells. This is because the response of individual cells is attenuated too greatly at the cornea to be measured, thus the ERG is composed of the mass response of a large number of cells whose responses are similar and add up to a much larger signal. The structure of the retina is such that only those cells with a radial orientation (receptors and Mueller cells) generate enough current at the cornea to be measurable (Rodieck, 1973).

The response of the receptors arises from modulation of a current which flows from the cell body and inner segment of the receptor to the outer segment. Figure 1B.1A shows the portion of the ERG which is due to the receptors. It is called the late receptor potential (LRP). The leading edge of the LRP appears in the ERG as the a-wave. There are two types of receptors, rods and cones. Rods are used in dim

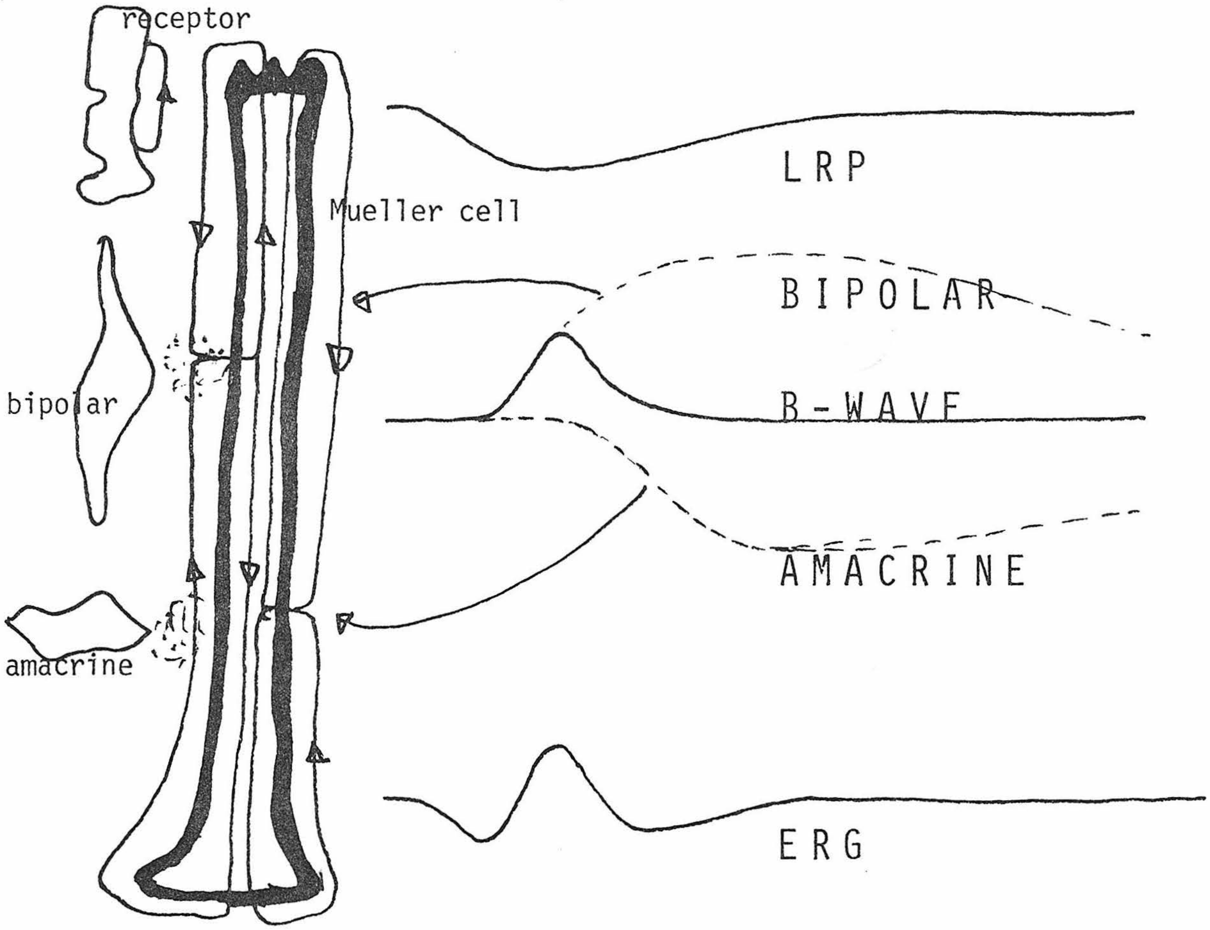


Figure 1B.1 The retina.

(scotopic) light and cones in bright (photopic) light, often responses from both are seen simultaneously.

The Mueller cells of the retina do not provide any direct neural function (Rodieck 1973), but mostly provide physical support and structure. It appears (Kline, Ripps and Dowling, 1978; Karwoski and Proenza, 1977; Newman, 1979) that the Mueller cells respond to increases in extra-cellular potassium ion concentration by generating a current from both of its ends to the part of the cell next to the extra potassium. However, the potassium concentration surrounding the Mueller cells increases when light activates the neural processing of the other cells of the retina. Thus, the Mueller cell currents are indirect references to the direct neural processing.

The cells which influence the Mueller cells most are the bipolar and amacrine cells (Kline *et al*, 1978). As shown in figure 1B.1B the two currents generated in the Mueller cells partially cancel, except for a relatively fast portion at the beginning which manifests itself in the ERG as the b-wave.

When the LRP from the receptors and the b-wave from the Mueller cells are added together to form the complete ERG (figure 1B.1C), they are superimposed and no longer are isolated components. A peak or a valley in the ERG is referred to as a 'wave'. The first wave in the ERG is a valley and is called the 'a-wave', the second is a peak and is called the 'b-wave'. The a-wave is the leading edge of the LRP and the b-wave is the uncancelled portion of the response from the Mueller cells.

1c -- systems and models

Models are formed for two basic reasons, communication and prediction. The use of models for communication of one's understanding of a system is something which is universal and is probably the most common use of models. Usually a model is an expression of the researcher's own intuitions about the system, formed through his experiences with the system.

One begins to use a model in a predictive fashion when one believes that it has reached a state where it is more than a mere representation of the facts at hand. For a model to succeed in this, it must not only account for known facts, but successfully account for phenomena beyond those which were used to generate the model. One very important use of a model is the correlation of the functional parts of the model with the underlying physiological structures.

The formation of a model of the adaptation of the ERG can begin with a system analysis approach. This is to apply a stimulus to the system and observe the response which it generates. Since the adaptation of the ERG depends upon many parameters of the stimulus, this usually requires the acquisition of many stimulus/response pairs with the parameters varied each time. The insights gained from this analysis then influence the formation of a model of the adaptation of the ERG. Although in a sense, these stimulus/response pairs are a mathematical model of the ERG and its adaptation, the design of the model is not necessarily mathematically oriented, nor are the stimulus/response data the only data which are incorporated into a model. Other data would be anatomical data and results from experiments on adaptation in animals where more direct methods are available.

A model of the human scotopic b-wave using system analysis techniques was formed by A. Troelstra and N.M.J. Schweitzer (1968). Their model and the underlying rationale will be developed here, then certain drawbacks will be discussed. This thesis is an extension of their ideas to photopic adaptation, and it requires the development of a more general technique.

Their model went through three phases of development, illustrated in figure 1C.1. The first phase consisted of a black-box which generates a response with the shape of a typical scotopic b-wave. This phase of their model could not predict the change in amplitude of the response to flashes of different intensities. However it did predict the shape of the response.

They found that the amplitude of the b-wave varied non-linearly with stimulus intensity when flashes of different sizes were used. This implies some sort of variable sensitivity mechanism; adaptation, although they did not refer to it as such. The next phase of their model added a variable sensitivity function before the b-wave mechanism to accomplish this (figure 1C.1B). They chose this order, because if the sensitivity function followed the b-wave generator, the shape would change with flash intensity. The non-linear sensitivity function was modeled by a multiplier which scaled the incoming stimulus by a stable non-linear scale factor. At this point the model predicted the response to a wide range of flashes of light. However, the model would not predict the response for stimuli which were preceded by other

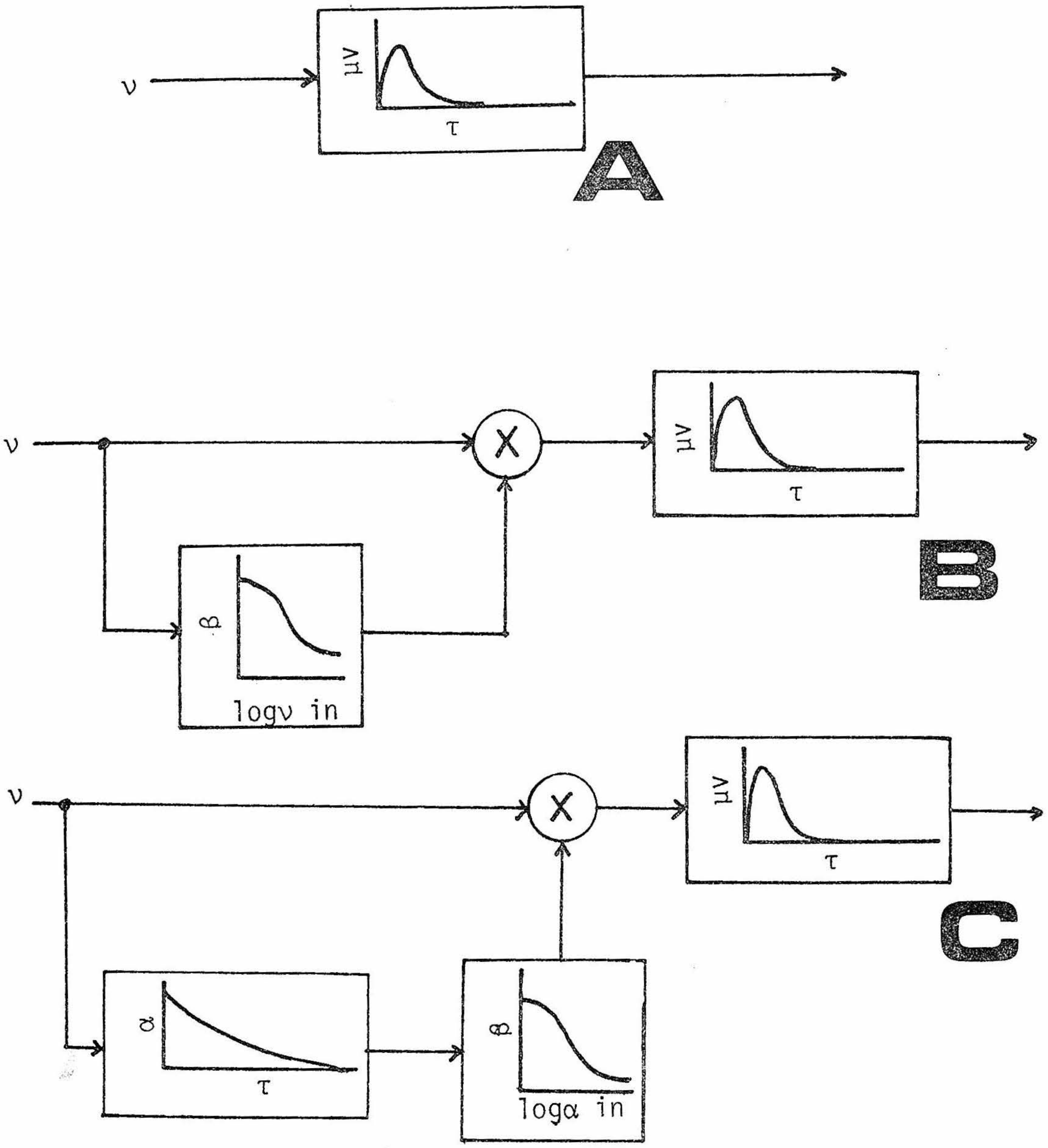


Figure 1C.1 The b-wave model.

The three phases of development of the human scotopic b-wave model of Troelstra and Schweitzer.

A) A simple linear model.
B) The addition to account for response compression.
C) The addition to also account for the time history function of the adaptation.

stimuli. The last phase accounted for this by adding a time history function to the scaling side of the multiplier (figure 1C.1C). This provided a weighted average of the intensity over the last second or so of the incoming stimulus and was used to scale the stimulus. The shape of this time history function was obtained by perturbing the system with one flash and waiting for a while before using a second flash to test the adaptation level.

To ascertain the generality of the model, it was tested with other stimuli such as steps, flash trains, sinewaves and flashes upon a background. They used their model to predict the responses to these stimuli, and in general it did quite well, not only with the shapes of the responses, but also in quantitative agreement with experiment. They also gave some ideas on how each of the components of their model might correspond to the physiological structures of the retina.

This model of the human scotopic b-wave gives a good idea of the type of model which can be used to describe adaptation, but it does not account for some important effects. One phenomenon which this model does not predict is suppression-recovery (Arden, Granit and Ponte,1960; Owen and Sillman, 1973; Salinger and Lindsley, 1972; 1971). When a flash train is presented, the response to the first flash is a given size and the model predicts this. This response to the second flash is smaller and the model also predicts this. However, the responses to succeeding flashes recover somewhat from the suppressed level of the second flash and the model does not predict this. A method for studying this phenomenon and its implications for adaptation are discussed in the results chapter.

Compared to the scotopic ERG the photopic ERG has rapid adaptation dynamics which occur "too quickly, in fact, to be accurately measured by the usual electroretinographic techniques." (Dowling, 1967) This applies to the model of Troelstra and Schweitzer, because their technique cannot measure the time history function of the adaptation for times which are shorter than the duration of the response to the conditioning (perturbing) flash. This is because the responses from the two flashes overlap and make measurement difficult. Although this was not a problem for the scotopic adaptation, the adaptation in the photopic ERG lasts for only about the same period as the response. Thus, some new method is necessary for characterizing the rapid adaptation of the photopic ERG.

1d -- impulse responses and kernels

Cross-correlation is a well-developed extension of the usual flash techniques which is well suited to the very fast nature of the adaptation of the photopic ERG. This technique usually uses a white noise stimulus which is also quite suitable for the characterization of the photopic rapid adaptation, although flashes and other traditional stimuli may also be used.

First, some of the reasons why flashes are one of the most used stimuli in the study of the ERG. A flash is a single, very short burst of energy which in general is called an impulse. Since an impulse is a single, isolated event, the response is also a single isolated event. Also, since the impulse is very short, the system is not affected by the past history of the stimulus. Thus the flash provides a simple stimulus for use with ERG's.

Next, some reasons to use cross-correlation and white noise instead of single (or double flashes) as a stimulus for studying photopic rapid adaptation. Photopic adaptation is studied by placing the retina in a photopic condition, then gathering essentially the same type of data which was used for the scotopic adaptation model. A fairly bright constant light is used to put the retina into a photopic condition. The pairs of flashes are then presented on this background. There are two differences between the responses in the photopic and scotopic conditions. First, the photopic responses are much smaller than the scotopic (roughly 10 microvolts vs. 100), and second, the adaptation takes much less time to recover its initial level (roughly 50 milliseconds vs. 1000).

Kernels are a method of processing and displaying multiple impulse data from cross-correlation analysis or flashes in such a way that the adaptation which occurs during the response to the conditioning flash may be seen. There are different orders of kernels which express different types of adaptation. The basic kernel, called the first order kernel (KF), is just the average impulse response of the system. The second order kernel (KS) expresses the type of adaptation which can be explored with two impulses, the conditioning impulse and the test impulse. The scotopic b-wave model can essentially be described with these two kernels. The suppression-recovery effects are expressed by the third order kernel (KT), which is obtained from three impulse experiments.

White noise is a type of stimulus which is good for photopic adaptation because it provides many stimuli in a short period of time which helps to average many of the small responses together for noise reduction. It also provides many different combinations of impulses for use in exploring the response relationships from two, three and more (if needed) impulses.

White noise and kernels are well established system characterization techniques which have not been well understood because they are usually associated with their complex mathematical background (Marmarelis and Marmarelis, 1978). Also, the form in which they have been traditionally displayed does not lend itself to easy interpretation, especially in regard to the higher order kernels (KS and above). In an attempt to bring a more intuitive approach to white noise and kernels, the next two chapters take each and explain the relevant features which are used to characterize the rapid photopic adaptation.

2) Stimuli
(What to look with.)

2a -- flashes vs. white noise

The question of whether to use white noise stimuli or flashes is not easily answered for one side or the other, it depends on the manner in which the system processes the stimulus. Here one encounters a further difficulty because the appropriate knowledge of the system is usually not available, thus requiring a certain amount of trial and error experimentation. Also, the choice between flashes and white noise is not the whole question. There are many other types of conventional stimuli which may be used for the generation of kernels, and there are many different types of white noise which have different characteristics and thus different applications. In light of this, the discussion of flashes vs. white noise should be taken only as a specific look into the question of stimulus choice.

Since the first order kernel is the average response to single flashes, it would seem that the use of white noise stimuli is an unnecessary complication. For simplicity's sake one might ask -- why use white noise at all? We will now discuss the various answers to this question, both affirmative and negative, and show that they depend on how the system processes the stimulus.

White noise stimuli and kernels were developed historically for the study of systems with dynamic non-linearities. The analysis of these systems can be accomplished with the use of the first order kernel augmented by the second (and even higher) order kernels. The white noise stimuli inherently provide the necessary ingredients for generating these higher order kernels, so they are very compatible with this form of analysis. This is not however, an overriding reason to choose a white noise stimulus because the same type of kernel may be generated from the responses to double (triple, etc.) flashes. The simplicity of the flash stimulus is essentially lost when multiple flashes are used, but their kernels contain the same type of information as those from white noise.

The main difference between flashes and white noise is their power and the distribution of that power within the time span of the experiment. Power is a measure of the fluctuation of the stimulus from its mean. Thus flashes, with their very small mean value, and their finite height, are very powerful stimuli. White noise, flashes presented upon a background, and flashes which are presented close together in a continuous train are necessarily low power stimuli. This is because they do not vary much further from the mean than the mean itself, so expressed as a ratio, their power is generally around one.

Flashes tend to be more powerful than white noise because of their isolated nature, but also because of that nature their power is distributed in individual events. Often this large power makes the flash the best stimulus to use, and if the response which is evoked by a flash is adequate, then flashes should be used. One system for which flashes proved to be appropriate was the scotopic b-wave system as measured by Troelstra and Schweitzer. However, this large power and its isolated nature can sometimes be a detrimental factor, such as in the blink reflex. When gathering data from human subjects one often records blinks which are evoked by the sudden appearance of a powerful flash. In this sense, white noise with its more uniform distribution of power, tends to evoke fewer blinks, and therefore is perhaps better.

When the response of a system is mixed with unwanted noise which requires signal averaging to remove, it is good to have a large power stimulus. The large power of the stimulus will create a large response which in turn will allow the signal to emerge from the noise sooner. It is usually desirable to have the experiments last for as short a time as possible, in our experiments this was because the subjects gave good responses for about a minute or two then blinked or moved their eyes or in some other way introduced noise into the record. Since the stimulus is presented for a certain amount of time, it is not the power of any given flash which is important, but rather the accumulated power of the whole stimulus over the experiment. In other words, a stimulus with a small power which is presented continuously (e.g. white noise) may provide a kernel which is just as clean as one provided by a few large flashes, (Marmarelis and Marmarelis, 1978, Chapter 7).

Some systems and some stimuli place a limit on the power of the stimulus which may be applied, in the photopic ERG the limit is placed because of the background. This limits the power because any stimulus must be referred to this reasonably bright level when measuring its power, rather than referring to darkness as with flashes.

Another system which has been studied with white noise because of peak power limitations is the study of gas exchange in breathing, where the stimulus is limited to the manipulation of the content of one breath at a time. A final example is the study of eye movements, where sudden movements of the stimulus cause loss of tracking, rather than a following movement. Thus the kernels from a continuous white noise are often better than those from a series of impulses (Williams, 1977).

The signal to noise ratio of the response also affects the choice of flashes or white noise. In general, it is advantageous to use flashes for very clean signals and white noise for signals which are deeply buried in noise. This distinction is based on two separate observations. White noise stimuli, being random in nature, introduce some noise of their own which must be averaged out with the response noise. This averaging occurs more or less simultaneously with the averaging of the response. The effect of this is that even if the response of a particular system is very low in noise, the white noise will still require the averaging of a fair amount of data because of the stimulus noise. So, if the system gives a response to one flash which was clean enough to use as such, it would be best to use that flash rather than white noise. However, if there was sufficient noise in the response to require averaging anyway, white noise is not necessarily at a disadvantage.

There is another aspect of a white noise stimulus which is advantageous when calculating high order kernels, i.e. second order and above. A white noise stimulus is inherently randomized. Thus any systematic variations over time in the system will not cause systematic errors in different parts of the kernels. This can happen easily when performing a series of double flash experiments, unless all of the individual pairs were intermixed randomly. Also, when using white noise, human subjects tend to be less aware of patterns in the stimulus which might affect attention or the response.

Thus, there is no distinct line between the use of isolated flashes or white noise for the stimulus in an experiment. Flashes tend to have the advantage of simplicity (of computation, generation and form) whereas white noise tends to have more subtle advantages (of power distribution and randomization) which for some applications offset its disadvantages.

White noise stimuli were used for the study of rapid adaptation of the photopic ERG because of several reasons. First, the multiple stimulus aspect of white noise stimuli is very well suited for the computation of high order kernels, the KS for linear adaptation and the KT for suppression-recovery. Second, when the background necessary for placing the system in the photopic regime is added to the stimulus, the power is dramatically reduced (note that although the intensity is higher, the power becomes smaller). With the low power stimulus which emerges, the continuous power level of the white noise provides quicker photopic kernels than flashes. And third, since the recorded signal from human subjects is not very stable over a period of a minute or so, (the gaze can wander, blinks can affect the signal, etc.) the randomization of the stimulus is very good at keeping the small effects of adaptation from being swamped by systematic differences due to longer term instability in the system.

2b -- white noise stimuli

There are many different types of white noise stimuli which may be used to study the ERG. We now use a 50% random flash, but the stimulus most used in the past has been gaussian white noise. These and other types of white noise are differentiated by their amplitude probability distributions and their generation statistics (figures 2B.1 and 2B.2). The choice of which to use is made based on their power, how easy they are to generate, and how easy it is to interpret the kernels which are obtained from them. Power is the ability of the stimulus to evoke a large response and is a good quality for a stimulus to possess.

GAUSSIAN

The 'traditional' white noise is gaussian, the stimulus for which the original theory was derived (Marmarelis and Marmarelis, 1978, chapter 4). The name is derived from the amplitude probability distribution of the stimulus. The gaussian stimulus has many properties which are helpful when used in the derivations of the normalization coefficients and the other theoretical aspects of this business. However, its low power is a drawback when used for studying the ERG.

The true gaussian has two characteristics which are not physically possible for light (photic) stimuli. The first is that a true gaussian distribution has a zero mean, i.e. it is balanced between positive and negative values. Since light cannot take on negative values, some compromise becomes necessary, usually a fixed background is added and the gaussian is generated around this. Thus positive values are above the background and negative are below. This however creates the second

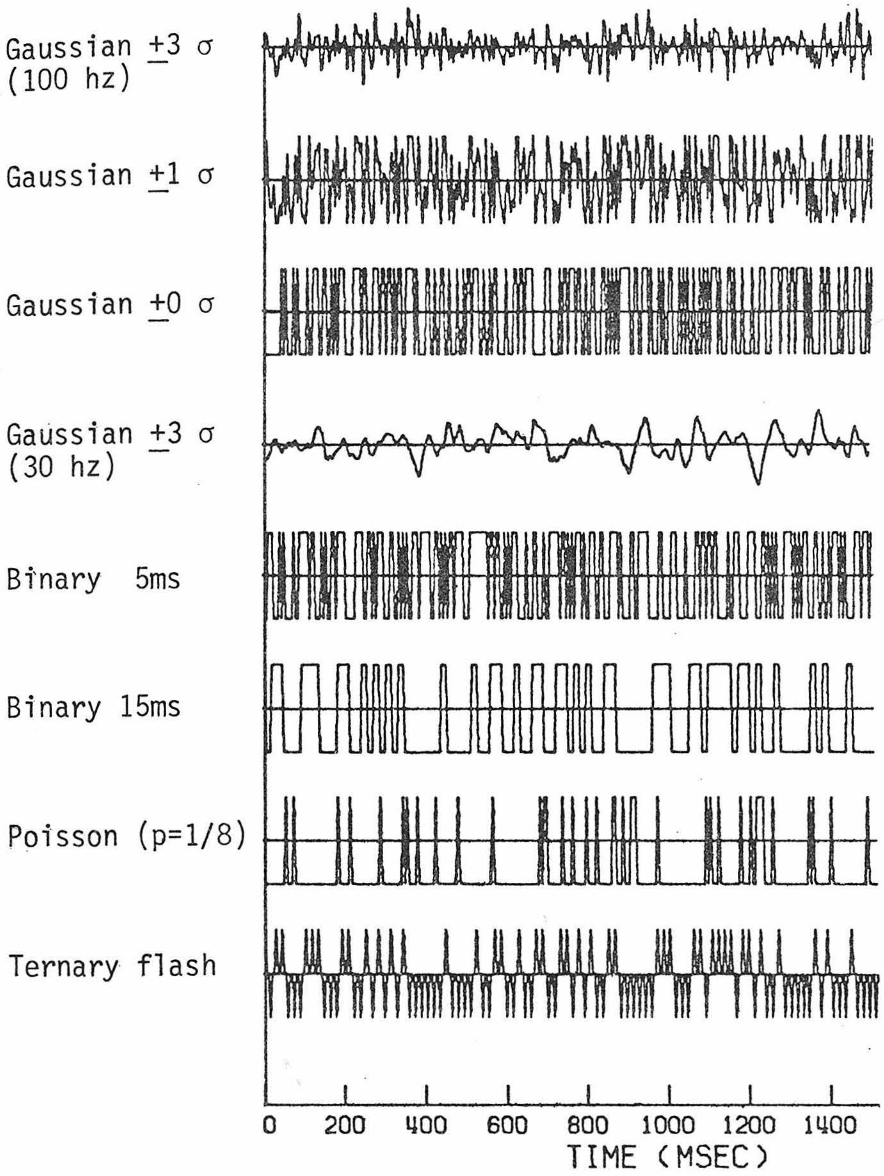


Figure 2B.1 Sections of white noise stimuli.

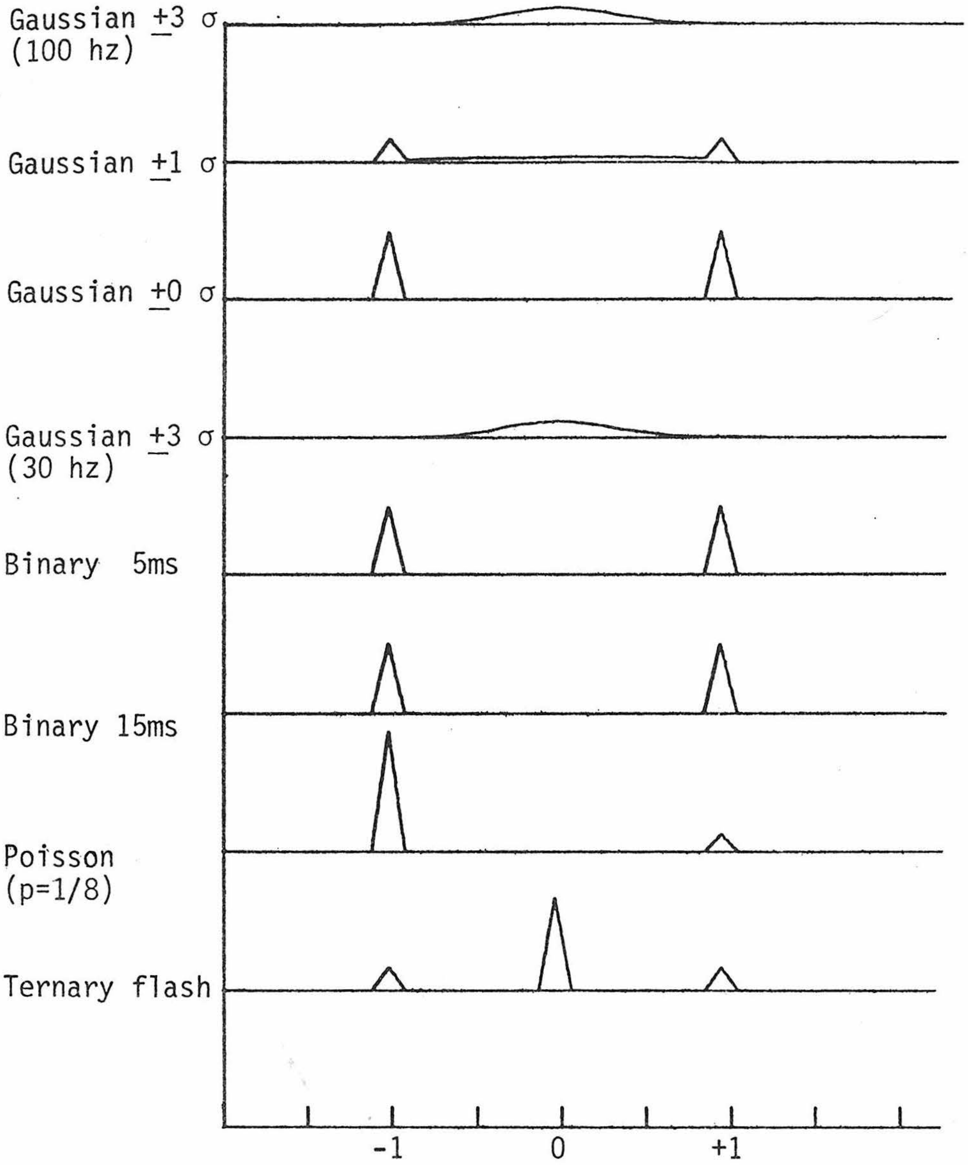


Figure 2B.2 Amplitude distributions of white noise stimuli.

The amplitude distributions for the stimuli of figure 2B.1. The ordinate is a plot of the number of samples which had the value of the abscissa. If the area under each of the curves is normalized to 1, the amplitude distribution becomes amplitude probability distribution. As described in the test, all of the stimuli have been scaled to a +1 to -1 range.

non-realizable characteristic, a true gaussian has an amplitude distribution which extends to infinity in both positive and negative excursions. Since the light cannot extend below zero, it must be truncated, and to preserve symmetry about the background level it must also be truncated at twice the background level.

The lower power of the gaussian is due to the fact that most of the time the stimulus fluctuates with a small amplitude. Since the distribution must be truncated to be used with a light stimulus, there is a method to increase its power at the expense of losing its characteristic gaussian distribution.

To allow comparison of the various white noise stimuli in as fair a manner as possible, all of their amplitudes have been scaled such that they do not exceed ±1. Thus, the truncation which will be applied to the gaussian will be at ±1. If the pre-truncation gaussian is scaled such that ±3 standard deviations is ±1, then the truncated (at ±1) stimulus is called a three sigma (sigma means standard deviation) gaussian. The amplitude probability distribution is still very nearly gaussian.

The amplitude before truncation may also be adjusted such that the truncation is at one standard deviation. The power is raised at the expense of a gaussian amplitude distribution. In the extreme case of an infinite amplitude before truncation, the truncated gaussian becomes a new type of stimulus, the binary stimulus, which only takes on the values of plus and minus one. It has been truncated at a very small value since it has infinite amplitude so it may be referred to as a 0 sigma gaussian or binary.

BINARY

The binary stimulus is the extreme case of the truncated gaussian. It sacrifices amplitude resolution (it has but two levels) for power. This is a good choice for the ERG which is noisy and benefits from the extra power offered by the binary stimulus. And the lack of resolution is not as serious as it may sound, this is not resolution in the kernel, but resolution of the stimulus.

The only important effect that this has is that the diagonals of the high order (KS and above) cannot be measured.

RAPID RANDOM FLASHES

The binary stimulus is a good stimulus to use for evoking the ERG. In looking for a way to easily generate a binary white noise, we began to use a strobe lamp. Our ERG response is sampled and recorded every 5 milliseconds (ms) so we generated a binary stimulus (5ms period), then either flashed the strobe if the binary was +1, or not flash it if the binary was -1. Since the ERG cannot distinguish two flashes 5 ms apart, the rapid random flash was exactly the same to the retina as a binary stimulus.

The rapid random flash stimulus is easier to understand by imagining a continuous train of flashes occuring at some frequency called the stimulus sample rate (which was 200hz in the last paragraph), and separated by the stimulus sample period (which was 5 ms). The random aspect of the stimulus is introduced by randomly deleting some of the flashes. The different types of rapid random flash stimuli are distinguished by the probability that a flash occurs, e.g. a 50% random flash stimulus is left with half of its flashes. There is another statistic, the average flash rate of the stimulus, which is the stimulus sample rate times the probability of a flash. Thus, for a 50% stimulus, the average flash rate is half of the stimulus sample rate.

The power of the rapid random flash (or any white noise) stimulus may be increased by sacrificing temporal resolution. This is done by increasing the stimulus sample period. We use a 15 ms stimulus sample period. This increases the power by a factor of three, but has the effect of only measuring every third cut of the KS. The name, rapid random flash, and a similar stimulus has been used in a study of the ERG by Fricker and Sanders (1975).

POISSON

If the probability of a flash is made smaller and smaller, but the stimulus sample rate is increased to keep the average flash rate constant, one approaches a poisson flash stimulus. This stimulus has good power and resolution, but is more difficult to use in the computation of the high order kernels because it is not a zero mean stimulus. However, it has great potential and should be considered, especially for those situations which only need the KF.

The advantage of flash stimuli over continuous stimuli is that they are more powerful. Their disadvantage is that it is somewhat tricky to measure actual luminous flux, and it is difficult to express it in a manner compatible with the kernel calculation methods.

2c -- stimulus measurement

For the ERG, a strong stimulus is not necessarily one which is bright, but instead one which flickers strongly. That is, a bright constant light does not generate any measurable response, whereas a dim light flickering at about 20 hertz generates a large response. Thus, contrast might be a better measure than intensity in this case. As in every aspect of system analysis, one must build on knowledge gained from any source, especially experience, in the decision of what system of measurement to use. The method of measurement should reflect the strength of the stimulus, which in turn reflects the ability of the stimulus to evoke a response.

One measures the stimulus in order to compare the kernels from different situations. Ideally, one system of measurement would be appropriate for all applications, otherwise one is forced to compromise and choose a measure which will only work for a subset of the whole problem. In looking at stimuli, it is important to use units for measurement which are compatible with the way that the system uses the stimulus, and which will allow comparison of the resultant kernels with appropriate generality. In the study of the ERG these two criteria are sometimes in conflict, for some applications the stimulus should be measured in terms of intensity, but for others the appropriate measure is in terms of the contrast (the variation from the mean) of the stimulus. This is not to say that sometimes the absolute intensity is not important to the system, it always is, but only that sometimes contrast gives results which are more intelligible.

The analysis of eye movements is another example of the necessity for proper stimulus measurement. The stimulus in this case is a dot which moves back and forth, and the response is the position of the eye of the subject watching the dot. As it happens, the proper representation of the stimulus is not the position of the dot, but instead the velocity (Williams, 1977). Thus, for some systems and some stimuli, it may not be appropriate to measure the stimulus in the obvious fashion, but some thought should also be given to the system.

2d -- equivalent impulses

The equivalent impulse is the auto-correlation of the stimulus (Appendix I). It is, to a first order approximation, the stimulus which, if applied as an isolated event, would generate a response which would look like the first order kernel of the system (figure 2D.1). This is a construct which allows one to interpret the kernels obtained from white noise stimuli in a fashion compatible with interpretation of conventional impulse responses.

Not only do equivalent impulses help in the conceptual interpretation of kernels, but the characteristics of the equivalent impulse of a stimulus define many aspects of the global stimulus. All stimuli, not only white noise, have an auto-correlation, and therefore have an equivalent impulse. Good stimuli have an equivalent impulse which is impulse-like, i.e. flat everywhere except at the origin, where they have a narrow spike.

The width of the spike corresponds to the temporal resolution of the stimulus, that ability to resolve events in the kernel which are very close in time. If the rest of the equivalent stimulus (everything other than the spike) is not flat, then that stimulus will introduce some distortion into the kernels (figure 2D.2). Having a narrow equivalent impulse which induces no distortions is equivalent to being white. White (as in white noise) means that the frequency content of the stimulus is uniformly distributed over a given finite band. A similar function is used to examine the effects of the stimulus when computing the higher order kernels, the second (and higher) order auto-correlation. An

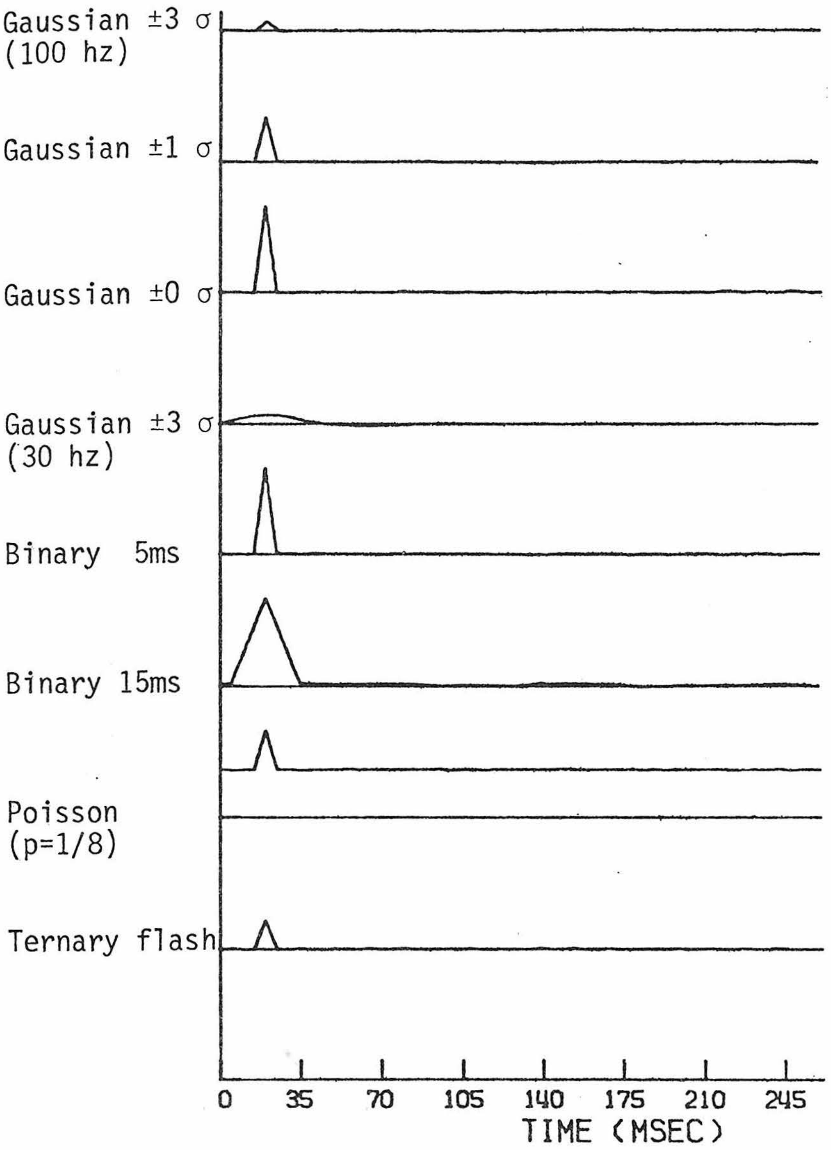


Figure 2D.1 Equivalent impulses of white noise stimuli.

A comparison of the equivalent impulses for a selection of white noise stimuli. All plots are on the same scale (1.5 microvolts between baselines). As before, all stimuli range between +1 and -1 microvolts. For a sample of each stimulus, see figure 2B.1.

The power of the stimulus is proportional to the area under the equivalent impulse. Notice how the amplitude and width of the curves are traded off for different versions of the same stimulus.

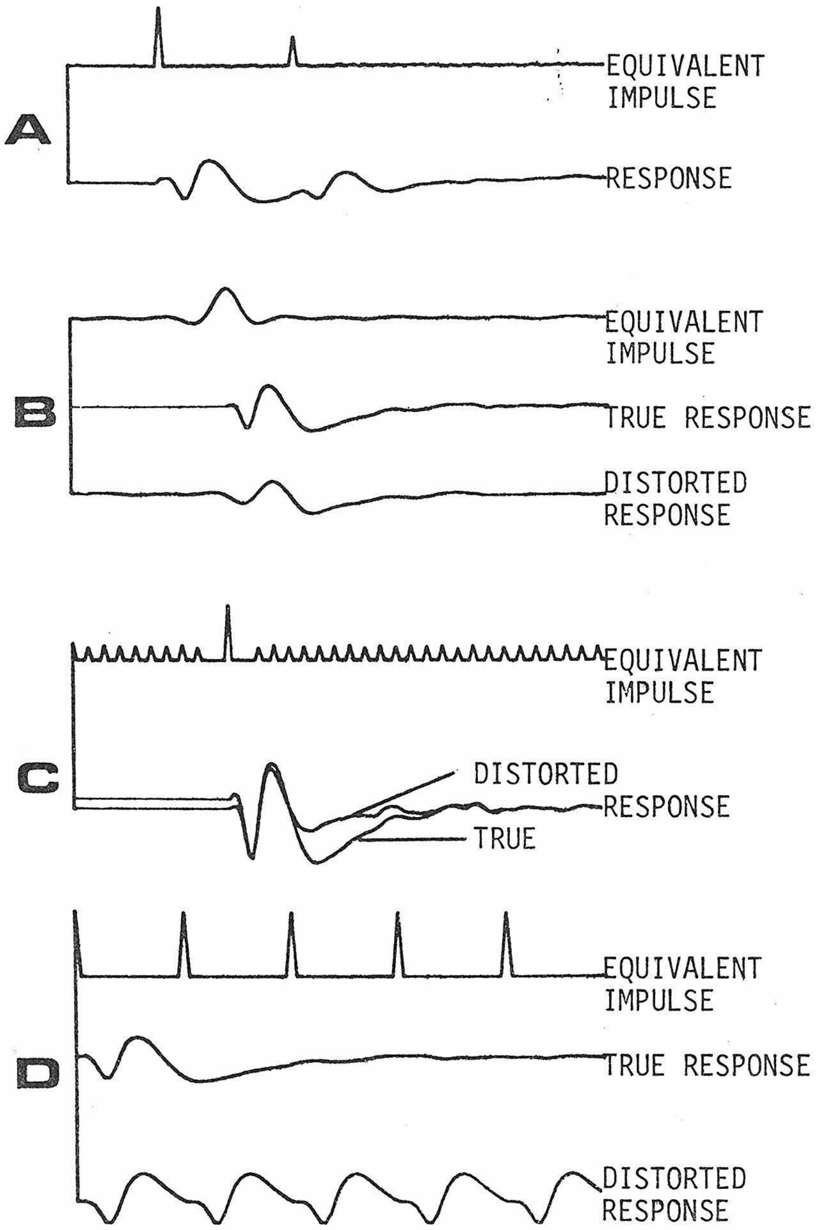


Figure 2D.2 Examples of poor stimuli.

A) A poor shift register sequence with unwanted correlations.
B) A band-limited gaussian stimulus.
C) A stimulus derived from triggering on the changes from zero to one in the sequence, rather than triggering on each 'one'.
D) A 10 hertz flash train. This is a typical flicker stimulus. Notice how the later parts of each response are not usable.

example of high order distortion is in figure 2E.1.

The area under the equivalent impulse corresponds to the power of the stimulus (Appendix I). To see the effects of any non-linearities, the power must be large enough to cause the system to use the non-linearities, and this usually means a large power stimulus. In the case of adaptation in the ERG, if the stimulus has low power the system does not adapt much. Thus the difference between a response from the system when it has adapted and a response when it has not, is very small. Since the kernels contain some noise, it can be very difficult to see the effects of adaptation when using a low power stimulus.

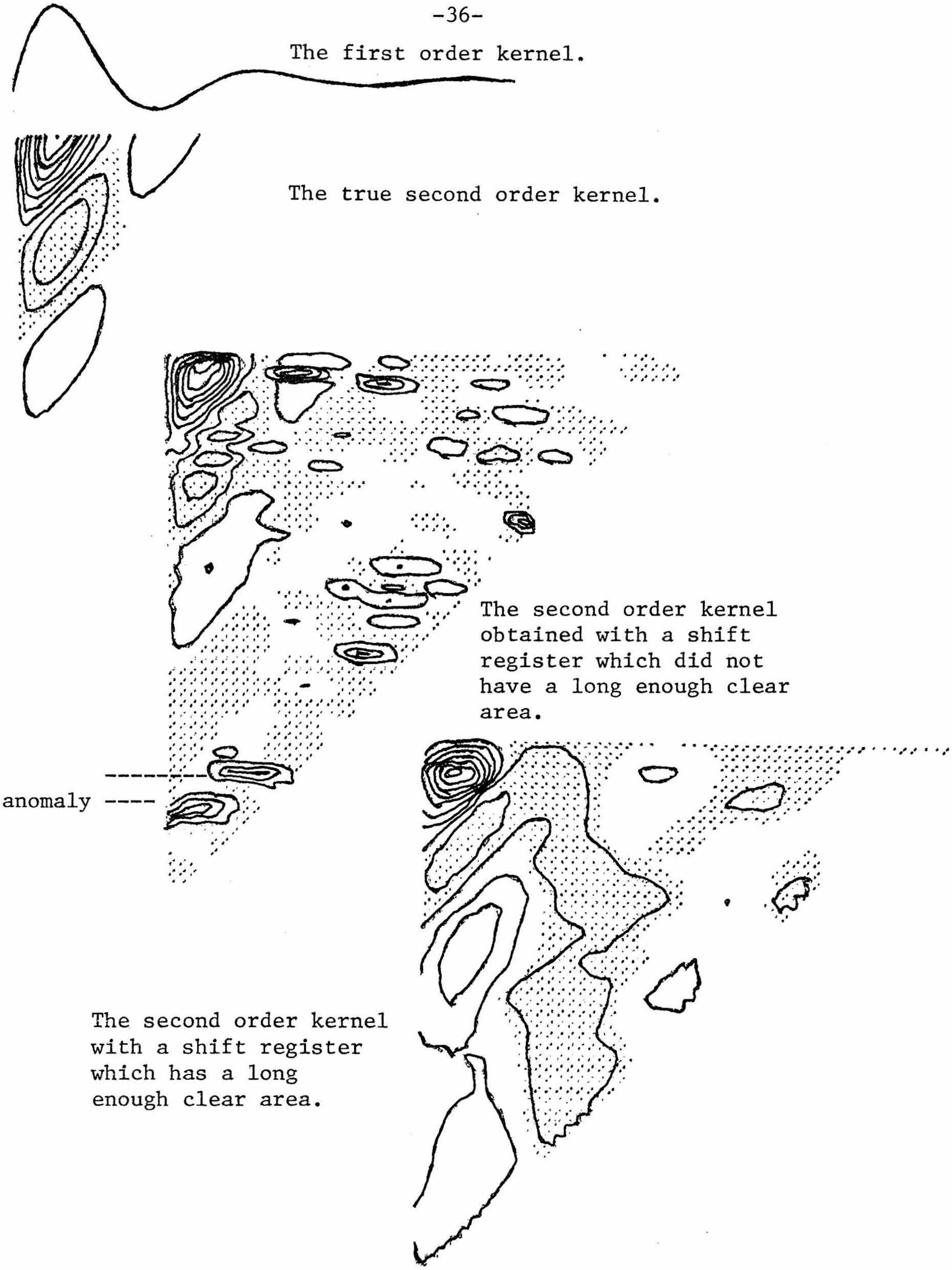
When restricted to a given amplitude range, each stimulus has a tradeoff between power and temporal resolution. The product of the power and the resolution is constant for any given type of stimulus. This power-resolution product is a figure of merit for comparing different stimuli.

2e -- shift registers

Once it has been decided to use a white noise stimulus it must be generated. Since the stimulus is random and therefore has no pattern, if is difficult to generate in most of the usual ways. The obvious technique of using naturally generated (truly random) noise has few advantages and is very difficult to generate properly (Marmarelis, V., 1973). Thus, more often the noise used is artificially generated.

Artificial noise may be generated either with a computer or a shift register. When a computer is used it must be available for real-time use during the experiment, or some provision for storing a computer generated sequence is needed. In light of these problems the use of shift registers to generate the stimulus is a common practice. The reason that they are not universally used is that when used improperly, they can introduce systematic errors which are subtle in source, but not in appearance, into the higher order kernels (figure 2E.1).

A shift register is an electronic device which holds a string of bits (a bit is one binary digit, i.e. a '0' or a '1'). This string of bits is shifted to the right by one bit at the stimulus sample rate, thus losing the old rightmost bit and gaining a new leftmost bit. The new leftmost bit is made a 0 or a 1 by looking at a combination of positions (which have been tabulated) of the shift register and counting the number of 1's which are in those positions. If the number is even, a 0 is shifted in, if the number is odd, a 1 is shifted in. If the combination of positions is chosen properly, the bits which come out (the rightmost bits) of the shift register will alternate between 0 and



The first order kernel.

The true second order kernel.

The second order kernel obtained with a shift register which did not have a long enough clear area.

The second order kernel with a shift register which has a long enough clear area.

Figure 2E.1 Improper shift registers.

1 randomly. This sequence of bits is then a binary white noise stimulus which will contain $2^N - 1$ random bits before repeating, thus a twelve bit shift register will generate 4095 random bits before starting over. More details on these registers is given in the following references (Ream, 1970; Barker, et al, 1970; 1972; 1973).

The problem of systematic distortion of the high order kernels comes from the regular nature of the generation of the sequences. The first order kernels from these stimuli are not affected by these anomalies, as they are called, however the higher order kernels can be. To allow the use of shift registers some method for avoiding these anomalies is needed.

These anomalies appear almost anywhere in the kernels, however there are clear areas in which they do not appear. A shift register is guaranteed to have a clear area equal to its length times the stimulus sample period, so it is possible to use this as a rough guide. There are shift registers with clear areas longer than this and they have been tabulated (Barker, et al, 1972).

Truly random, and most artificial sources of white noise have inherent noise which is reduced proportionally to the square root of the length of the time which has been averaged. The shift register sequences have this same behavior for the first part of a cycle, but at the middle of the cycle the amount of accumulated stimulus noise starts to decrease, and reaches zero at the end of the cycle. This means that using these types of stimuli over a complete cycle results in lower noise in the kernels compared to other types of white noise.

Therefore a tradeoff exists between the choice of a long shift register and a short one. Longer shift registers give longer clear areas free from anomalies, but short ones are able to complete a cycle during the experiment, thus providing low noise. Through experience we have found that a 15 ms rapid random flash stimulus offers a good tradeoff between power and high order kernel temporal resolution. We have also found that 250 ms is a good length of time for the high order kernels. When we decided to use a shift register for generating our stimulus, we wanted to avoid anomalies so we made our register 17 bits long (15 times 17ms = 255ms). The cycle time of this sequence is about a half hour, which is much too long for an experiment. Thus we have foregone the extra advantages of using a whole cycle in favor of avoiding anomalies.

3) Kernels
(How to look at systems.)

3a -- interpretation of kernels

The average processing of the stimulus by the system is reflected by the first order kernel (KF). The KF is calculated by the process of cross-correlation (appendix I). As discussed earlier, it is analogous to the average flash response of conventional methods, but has the advantage that it not only can express the average flash response, but also the average response to a white noise stimulus (figure 3A.1).

The second order kernel (KS) shows the average change in the KF when preceded by a conditioning stimulus. The conditioning stimulus is an equivalent impulse, just as the test stimulus is an equivalent impulse. Since the change in the KF will be different depending upon the time between the conditioning stimulus and the test stimulus, the KS has an additional dimension beyond the KF (figure 3A.2). The abscissa is the same as for the KF, time after the test stimulus, but now the ordinate is the time between the conditioning stimulus and the test stimulus. The information which corresponds to the ordinate of the KF is expressed by the values of the contours in the plot of the KS. Since the KS expresses the change in the KF due to a conditioning stimulus, it begins to give an idea of what the effect of adaptation is upon the system. Because of the close relationship between the KF and the KS, it is imperative that the KF be displayed with and aligned with the KS to allow proper interpretation of the KS.

In a like manner, the third order kernel (KT) shows how the KS is modified by a second conditioning stimulus in addition to the first

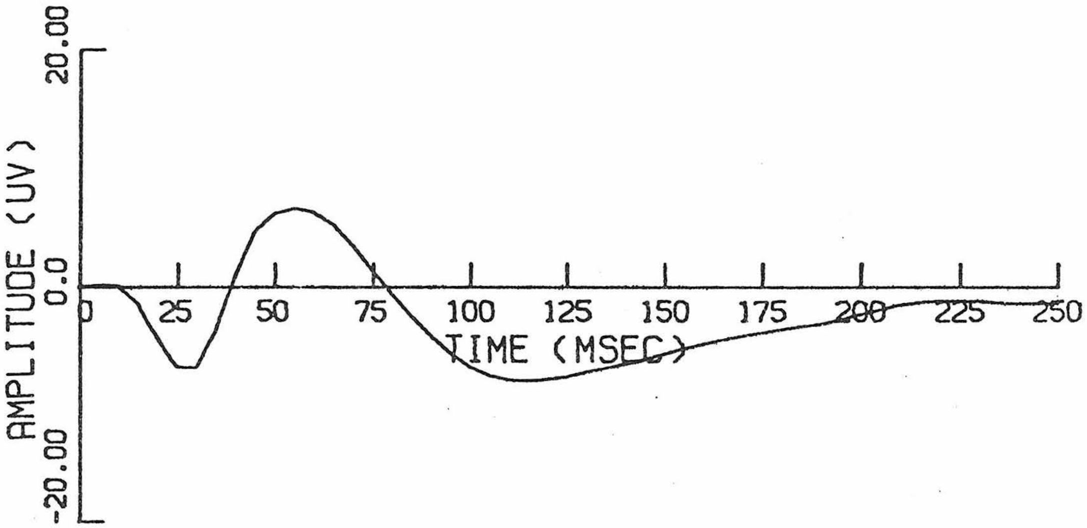


Figure 3A.1 A typical first order kernel (KF).

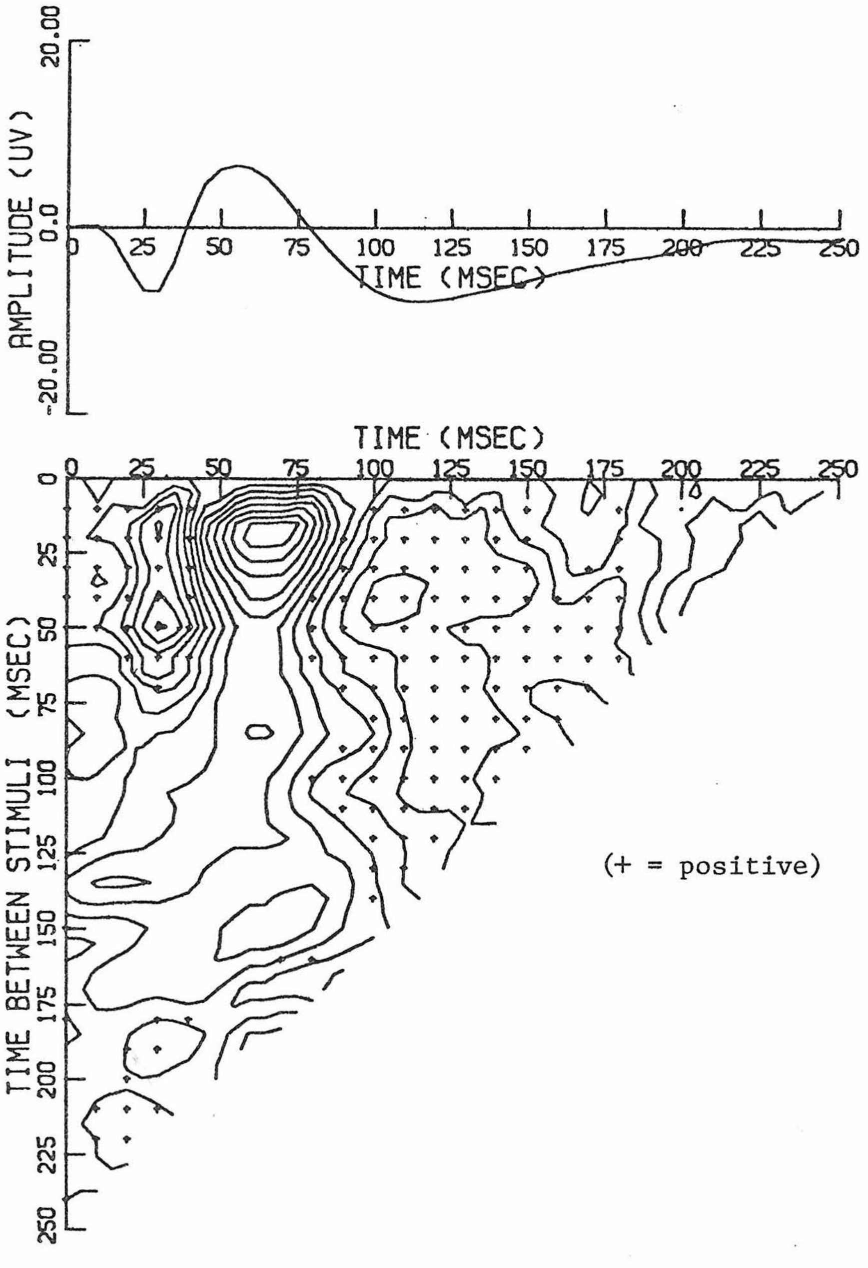


Figure 3A.2 A typical second order kernel (KS).

This is a plot of the first and second order kernel properly displayed together (in mode 2, M2). The abscissa for each is aligned for comparison of features between the kernels.

conditioning stimulus. To express the entire KT required a four dimensional space which is, needless to say, difficult!

The ERG and other systems, by virtue of the type of processing that they do, theoretically require the use of an infinite number of kernels to completely describe their inner workings. This sounds discouraging, but often the effect of each additional kernel is less than the preceding one. Also, with the other information about the system which the researcher has, it is usually possible to see a pattern which makes it unnecessary to go past the KS or KT.

DISPLAY

The kernels have a definite form which must be displayed properly to give an easily understood and relevant indication of the operation of the system. The information displayed in the KS and the format of the display of that information offer many different possibilities. First the question of what to display. Since the type of information is that provided by a double impulse response, the options are: 1) the double impulse response as such, 2) the double impulse response with the response to the conditioning stimulus subtracted off, or 3) the double impulse response with the average impulse response subtracted off from both the conditioning and the test impulses (figure 3A.3). The last option is the one which is usually used for the display of the kernels. It displays only the effects due to adaptation without the (usually much larger) KF superimposed.

Once the information to be displayed has been decided, the format must be arranged. There are again three different methods, designated mode 0, mode 1 and mode 2 (M0, M1 and M2). Basically, the three differ

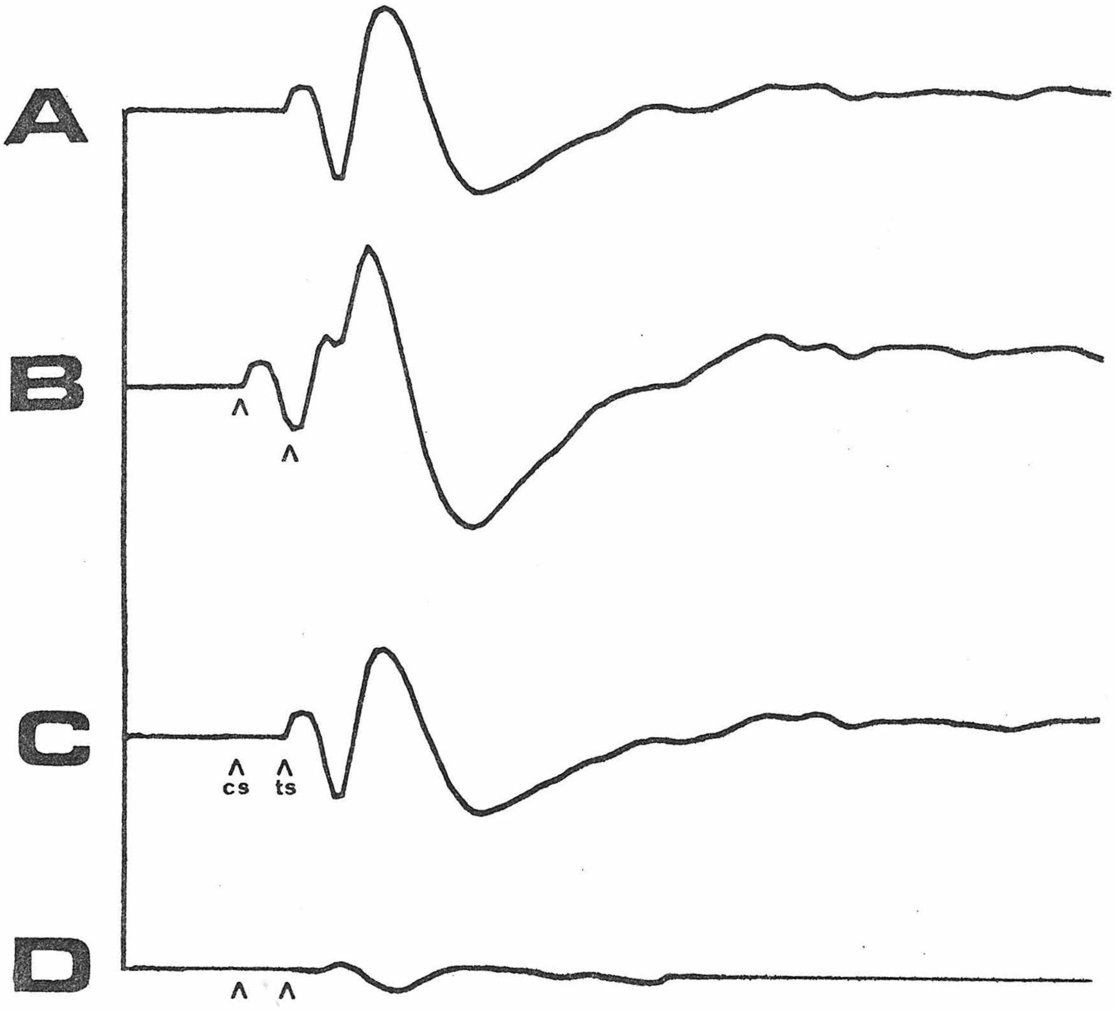


Figure 3A.3 The information in the KS.

A) The KF.
B) The two impulse response.
C) The two impulse response with the KF subtracted from the conditioning stimulus (cs).
D) The two impulse response with the KF subtracted from the conditioning stimulus (cs) and the test stimulus (ts), leaving only the effects of adaptation.

Note that it is quite difficult to see the difference between C and A, but the difference is easily seen in D.

on their point of reference, M0 refers to time from the response, M1 - time from the conditioning stimulus, and M2 - time from the test stimulus (figure 3A.4). The first mode, M0, is the historical format which has been used in the past, but which is difficult to interpret. It is symmetric about its main diagonal (higher order kernels have other diagonals in addition). Modes M1 or M2 are used to display the KS for a simpler interpretation. In both modes the main diagonal is at the top of the kernel, and is not actually a diagonal in these formats. The choice of M1 or M2 is made based upon the organization of the system (see section 5d). The ERG is best interpreted using M2, which will be used throughout, except for section 5d.

INTERPRETATION

When a KS for the ERG is displayed in M2 it fortunately has a form which is amenable to a reasonably simple interpretation. Cuts through the KS have essentially the same shape as the KF, merely inverted, smaller and slightly time shifted (figure 3A.4, M2). Each component of the ERG is somewhat differently represented in the KS cuts because of their different adaptation dynamics. But the fact that the KS cuts are similar to the KF simplifies the analysis immensely.

The fact that the KS is inverted when compared to the KF means that the adaptation of the ERG suppresses the response to a test flash which follows a conditioning flash. Note here that suppression (as opposed to enhancement) is determined by comparing the polarity of the KS to the polarity of the KF, not by merely examining the polarity of the KS alone. The relative size of the KS when compared to the KF tells of the magnitude of the adaptation. And the shift (away from the beginning of the

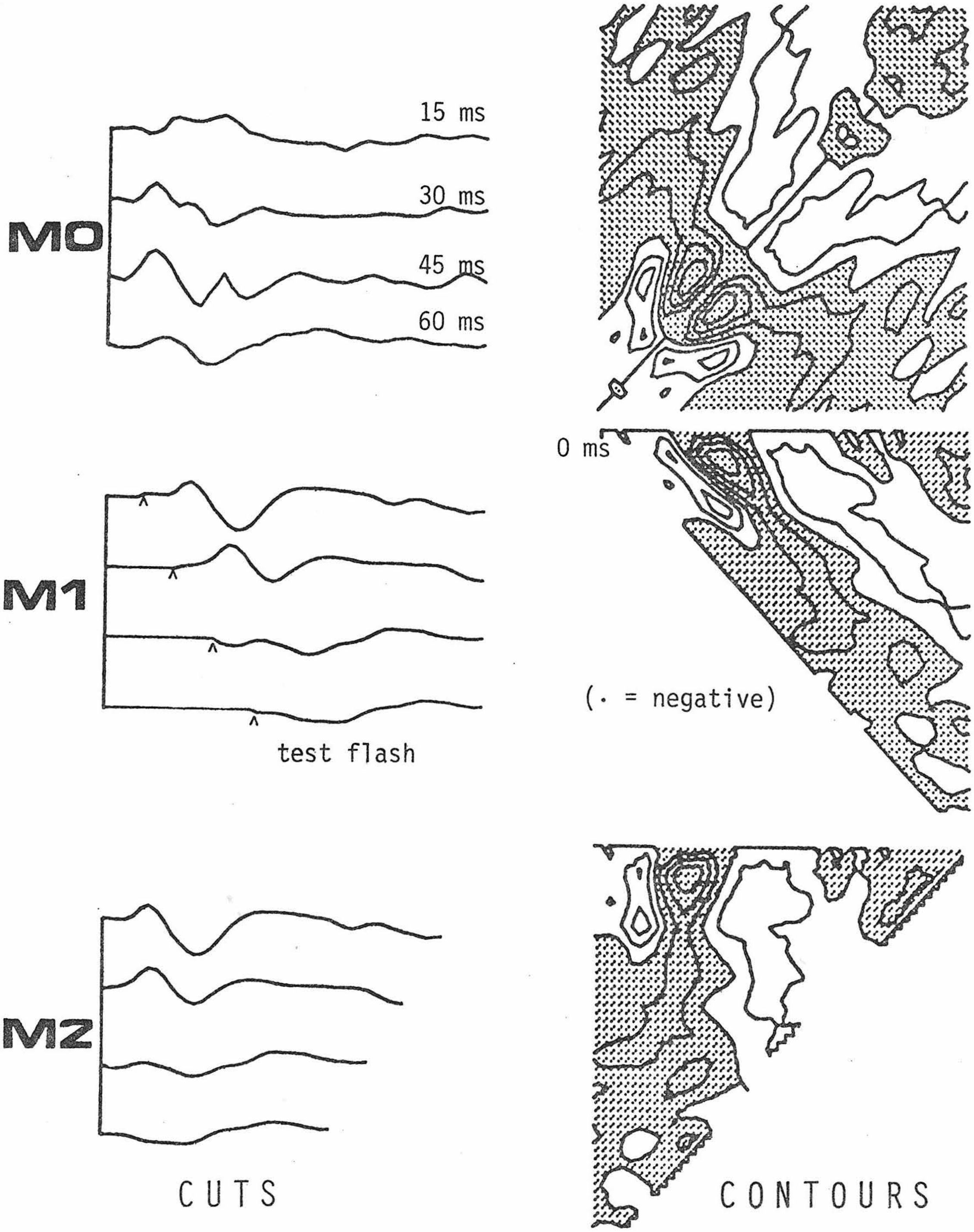


Figure 3A.4   Modes of display of the KS.

All of the second order kernels (KS) displayed in this figure are the same, the only difference is the mode of display.

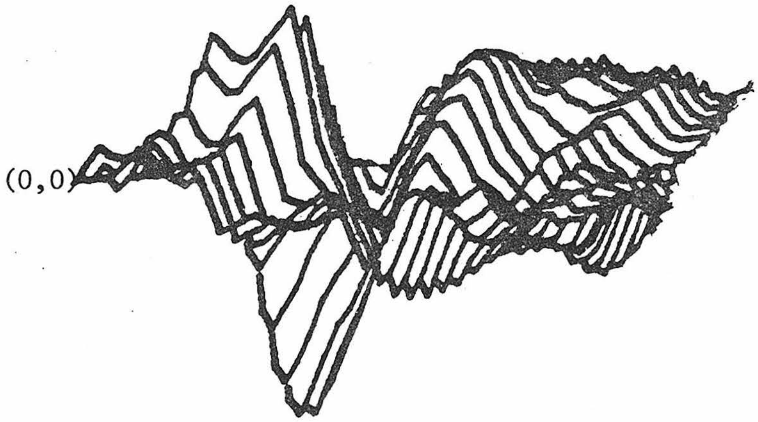


Figure 3A.5 Relief plot of a KS.

Often the second order kernel (KS) is plotted as a 'perspective' view of an equivalent surface. This is an alternative to the plots of cuts or contours of figure 3A.4.

This is the same KS which is plotted in figure 3A.4, from a position corresponding to the word CONTOURS. To the left, the peak corresponds to the positive area close to the origin; below that, the valley corresponds to the negative contours.

This method of displaying kernels has great visual appeal, but is nearly impossible to use. First order kernels are not easily compared to the KS this way, nor are KS's easily compared to each other.

kernel) means that a bright stimulus which precedes the KF will not only suppress the KF, but slightly speed it up.

As in conventional responses of systems to various stimuli, there is often confusion in the proper interpretation of kernels because of the presence of multiple components in the system response. The ERG is, for example, composed of mainly the LRP and the b-wave, seen as the a- and b-waves. Any interpretation of the KF (or the conventional average flash response) must take these separate components into account. These same components also appear in the higher order kernels, but since they do not each have the same adaptation dynamics the interpretation of the KS may be further complicated.

LINEAR ADAPTATION

Adaptation as characterized by only the KF and the KS is symmetric, the amount of adaptation caused by a positive impulse is the same (but reversed in polarity) as that caused by a negative impulse. Also, the adaptation which is caused by two flashes together is the same as that caused by each separately but added together. The adaptation reflected in the KS thus follows the principle of superposition, and is thus linear.

The term 'linear adaptation' seems contradictory, but it does not mean that adaptation is linear, adaptation is by definition non-linear. It instead refers to the characteristics of adaptation as reflected in the KS. The KF reflects the linear aspects of the system because it only depends on the response to one impulse. In the same manner, the KS refers to the linear aspect of adaptation because it expresses the behavior of adaptation caused by one conditioning stimulus. The aspects of adaptation which are expressed in the KS have similar features to those of the response expressed in the KF.

A MISCONCEPTION

A popular misconception in the interpretation of the KS from a system is that it expresses the response of the system to all possible double impulse interactions. This is not true, the KS expresses only a small subset of the possible double impulse interactions, only those between two identical impulses. This has implications in the interpretation of all elements of the KS.

The diagonal elements of the KS express the effects of two impulses which are so close together that they are considered one impulse of twice the height of the equivalent impulse of the stimulus. The main diagonal of the KS is the difference between the response of this double height impulse and the responses to each of the impulses alone added together. Thus, if the system were linear, the main diagonal (and the rest of the KS) would be zero. The misconception is that the main diagonal somehow expresses the non-linearity involved in the response to an impulse of any height. This is not true.

A similar misconception exists about the interpretation of the off-diagonal elements of the KS. There is no information on the effect of a double height impulse on a single height impulse, for example. This effect is expressed in one of the auxiliary diagonals of the third order kernel.

3b -- kernel scaling

Once one has calculated a KF and a KS, how are they compared to each other? How are they compared to kernels obtained from other stimuli, or even kernels from the same type of stimulus, but at a different intensity? These questions have two parts, the first is -- what are the proper units for the kernel? And the second is -- after the units have been determined, what normalization factors are involved?

The units of the KF are simply the units of the response divided by the units of the stimulus. The units of the KS are the units of the response divided by the square of the units of the stimulus, and so on for the higher order kernels. There is no single method for the measurement of kernels which is universally applicable. In fact the first conflict has already arisen, i.e. the units of the KF and the KS are not the same. This means that the KF and the KS are not directly comparable numerically. To resolve this conflict, one must decide what one wishes to compare. Basically, there are two methods of kernel measurement which have been used, the conventional flash method and the white noise method. These names refer to the stimulus historically associated with each technique, not to the stimuli with which they may be used profitably.

CONVENTIONAL FLASH METHOD

When one wishes to compare kernels of different orders to each other, e.g. the KF to the KS or the KT, the method derived from the conventional flash method is used. This method basically leaves the stimulus out of the units and the measurement. In the ERG, the stimulus used is light and the response is the electrical signal generated. Thus, the units of the KF are (unit of electrical measurement) per (unit of light measurement). The appropriate unit of electrical measurement is obviously microvolts. The appropriate unit for light measurement when using the traditional flash stimulus has simply been the 'flash', with reference to its size not being in the units, but instead stated as a separate fact. Kernels measured in this fashion have units of microvolts/flash, or in effect just microvolts, since the flash is simply a scalar value

To generalize this method to the measurement of the kernels, one must use the idea of the equivalent flash of the stimulus. Using this in the same manner, the units of the KF are microvolts/equivalent flash, or as before, just microvolts. Since the units of the stimulus have thus disappeared, the units of the KS and in fact all orders of kernels are microvolts. Thus all orders of kernels are comparable in this system.

WHITE NOISE METHOD

When one wishes to compare kernels obtained at different intensities, at different contrast levels, or with some other parameter changed, the method historically associated with white noise works well. It is basically the same as the conventional flash method, but with the units, and therefore the power of the stimulus left in the formula.

This method reflects the fact that the response to a small stimulus will be smaller than the response to a large stimulus, even though the characteristics of the system may be exactly the same for both stimuli. This is compensated for by dividing the KF by the power of the stimulus (appendix I). This scales the KF from small stimuli up to match the KF from large stimuli. For an example of the two methods see figures 5A.5 and 5A.6. The KS is scaled by the power squared to allow comparison at different power levels too, but it then is not comparable to the KF.

NORMALIZATION

There is a factor of two difference when comparing kernels to flashes as opposed to traditional white noise kernels. When using random flash stimuli at a high average flash rate, as is done with white noise, the stimulus provides an average intensity of light which acts as an effective background. The stimulus is then measured, not as flashes on darkness, but as flashes above the background or as 'no flashes' which extend below the background. Thus, the measurement provides a zero mean stimulus compatible with the white noise analysis. The representation of the random flash white noise stimulus is a +1 whenever a flash has been delivered, a -1 when a flash could have been delivered but was not, and a 0 when a flash could not have occurred (Our stimulus sample rate is 15 ms with a 5 ms data sample rate, thus two out of three sample periods cannot have a flash and will be zero.)

When using this representation, however, a choice must be made on whether to call a non-flash a stimulus or not. That is, when viewed as a true white noise stimulus, the -1 (a non-flash) is just as valid as the +1 (a flash) in the analysis, whereas usually a non-flash is not considered a flash and is measured as a zero. The only effect this has is that counting non-flashes as flashes give kernels which are half the amplitude that they would be if non-flashes were not counted.

Either of these normalization methods may be used with either of the measurement methods. We use the +1, -1,0 method unless the kernels are compared to average flash responses when the +1,0,0 method is used.

3c -- radius of convergence

One of the uses of kernels is to try to predict how the system being studied will respond to a stimulus (the 'imaginary' stimulus) other than the stimulus used to generate the kernel (the 'real' stimulus). As a first approximation the system can be approximated by the KF to see how it would respond to the imaginary stimulus. To do this one uses a process which is the opposite of cross-correlation, called convolution, (appendix II) which takes an imaginary stimulus and the kernel and uses the two together to generate an approximation to what the system itself would give as the response to that imaginary stimulus. If the real stimulus used to generate the kernel is similar to the imaginary stimulus of the prediction effort, then the predicted response will be similar to response which the system itself would have actually generated. As some parameter of the imaginary stimulus differs more and more from that of the real stimulus, the predicted response will differ more and more from the real response which would be generated by that stimulus. One major reason for this difference is adaptation. For instance if the imaginary stimulus differed from the real one by being brighter, the predicted response would be too big because the real system would adapt and decrease the size of its response.

The amount that a given parameter of the imaginary stimulus can differ from that of the real one and still allow the KF to properly predict the response (within some arbitrary error) is called the radius of convergence of the KF with respect to that parameter. In the example of the adapting system, if the KS is also taken into account, the radius of convergence with respect to brightness will increase. This is because the effect of increasing the brightness is predicted by the KS. After some (larger) difference however, even the KF and KS together will not be able to properly predict the response which the system would actually generate. Thus even though the radius of convergence would be increased, it would still be limited.

The radius of convergence is a measure of the applicability of the kernels in modeling the operation of the system under study. It also, in general, increases with the addition of more kernels. The popular misconception that one set of kernels (KF, KS, etc.) will model the response of the system for any stimulus is thus equivalent to saying that the radius of convergence with respect to all parameters involved for that set of kernels is infinite. From experience it can be said that this is never true for biological systems.

3d -- power distribution

The ability to tailor a stimulus to distribute power to those aspects of a system which are of interest, at the expense of other parts which are not, is a definite challenge in the design of white noise (and conventional) stimuli. When one speaks of the stimulus distributing power into a part of a kernel, what is actually meant is that the stimulus, when applied, will allow that part of the kernel to change. The more power distributed to that part, the more often it will be affected. Since the signal to noise ratio for each part of the kernel goes up with accumulated power, and the power applied to a given part of the kernel depends on whether the stimulus puts power into that part, the signal to noise ratio of different parts of the kernel can be different depending upon the stimulus.

As an extreme example of power distribution, take the flash stimulus (random or not), which has only one size of flash (two levels, flash or no flash, i.e. binary). There is no information in the response about what the system does with double size flashes, they simply do not exist. Thus, binary stimuli do not accumulate any power on the diagonal of the KS, and this is why the diagonal remains undefined. But, binary flash stimuli offer resolution in the 'time' axis equal to the rate at which the response is sampled, and resolution in the 'time between stimuli' axis equal to the stimulus sample rate. If a KS is noisy when using a binary stimulus which spreads its power evenly over the KS, perhaps the KS could be improved with a different stimulus sample rate. A binary stimulus with a lower stimulus sample rate will only provide information on a few KS cuts, but they will be cleaner, and perhaps more meaningful than a whole, but noisy, KS.

Another example is a ternary stimulus which has three possible amplitudes and can therefore be used to measure the diagonal of the KS. The probabilities for each of the three levels can be manipulated to adjust the relative amounts of the stimulus which is distributed to the diagonal and off-diagonal elements of the KS.

These are some examples of adjusting the amount of the stimulus power which is distributed to various different parts of the KS. The power of the stimulus to a large extent determines the amount of the stimulus which is distributed to the various orders of kernels. We have not performed experiments to show this directly, however an experiment has been done with sine waves which may be used to demonstrate this phenomenon.

First, the power of a sine wave is proportional to its depth of modulation for a given average intensity. Second, the harmonic content of the response to a sine wave is related to the different order of kernels. The size of the fundamental in the response is analogous to the power distributed to the KF, the size of the second harmonic is related to the KS, etc. So it is possible to see how power affects the power distribution of the stimulus relative to the various order of kernels. The amount of each harmonic present in the response of the frog ERG has been determined for a number of different depths of modulation (figure 3E.1, from Troelstra, 1971). As can be seen, the higher the power, the higher the harmonic to which power is distributed. Likewise, with higher power more power would be distributed to the higher order kernels.

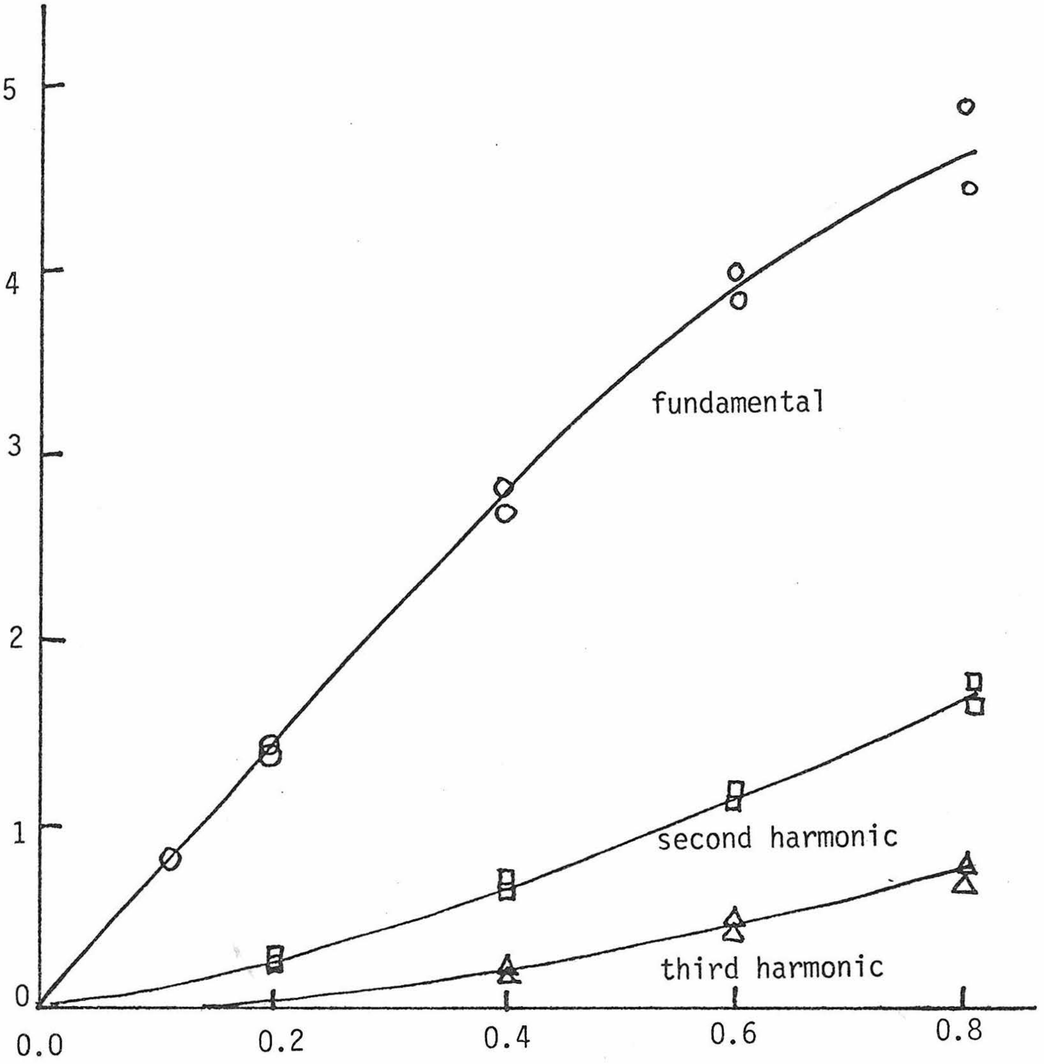


Figure 3E.1 Power and depth of modulation of a sinewave.

This figure (from Troelstra; 1971) displays the relative amounts of power which are distributed to the harmonics of the response. The abscissa is depth of modulation, the ordinate is relative amounts of power present in the response.

Notice that the relative amounts of each harmonic changes as the depth of modulation, and therefore the power, increases.

4) Experimental Techniques
(How to measure the ERG.)

4a -- equipment

STIMULATOR

We have decided, through experience with many different types of stimulators to use a xenon flash unit (Strobex model #136 with a model #70 head Chadwick - Helmuth Co., Inc.). This was used to present a rapid random flash stimulus (figure 4A.1) or a conventional slow flash to the subject. The flash was presented as a large field, free view stimulus. The large field reduced the problem which we have had with scattered light. When a bright, small field is used the area of the retina which is directly illuminated responds photopically, but the rest of the retina will adapt and respond to the scattered light scotopically. The scotopic response from the scatter is added to the photopic part of the ERG and makes interpretation difficult.

GOGGLES

The ERG is a potential which is generated between the cornea of the eye and the rest of the body (section 1b), thus some means of making electrical contact with the cornea is necessary. The traditional method for doing this has been to use a contact lens which has an electrode embedded into its surface. The type of contact electrode which is used at Doheny is the bipolar Burian - Allen lens (Burian and Allen, 1954). This lens has both the active and the reference electrodes embedded in it. The Burian-Allen lens also has a speculum around it to hold the eyelids apart during the experiment, to minimize the effects of blinks.

Associated with the Burian-Allen lens is a certain amount of discomfort, so we have developed a new type of electrode known as the

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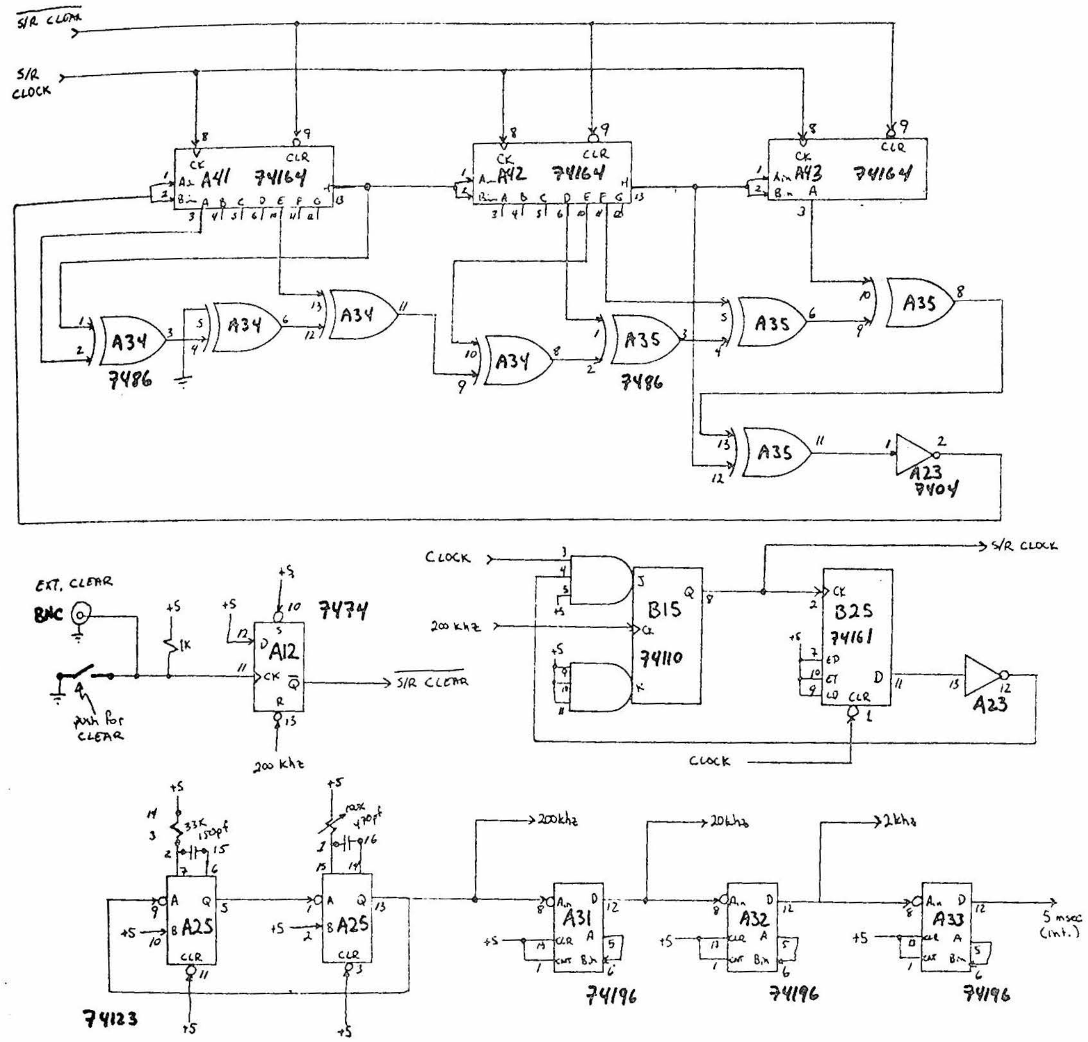
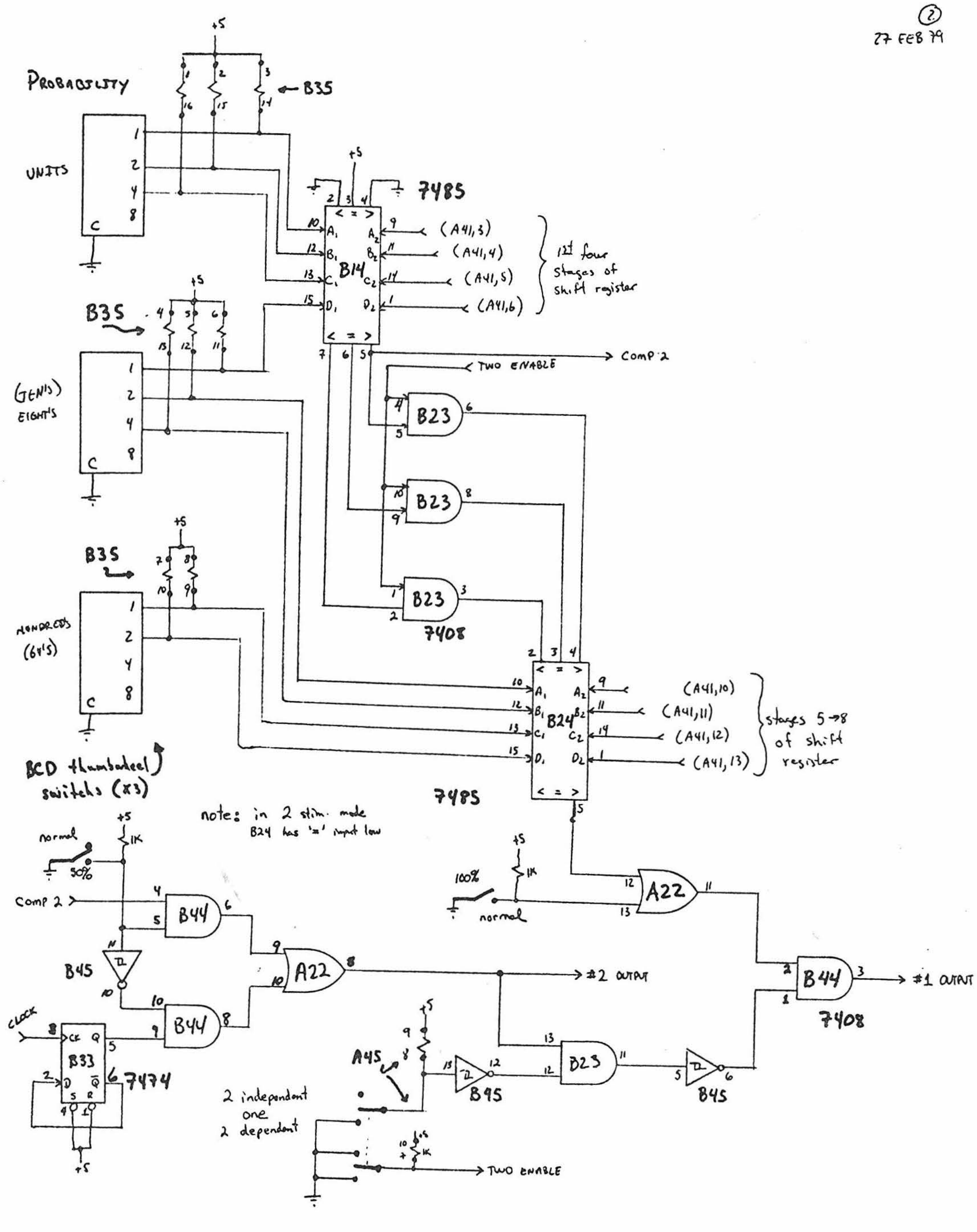


Figure 4A.1$_1$ The binary random sequence generator.

The shift register and feedback network (A34, A35, A41, A42, A43).

The clock generation circuitry (A25, A31, A32, A33, B15, B25).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note: in 2 stim. mode B24 has '=' input low

Figure 4A.1$_2$ The binary random sequence generator.

Probability determination (B14, B23, B24).

Output #1 vs. output #2 logic (A22, B23, B33, B44, B45).

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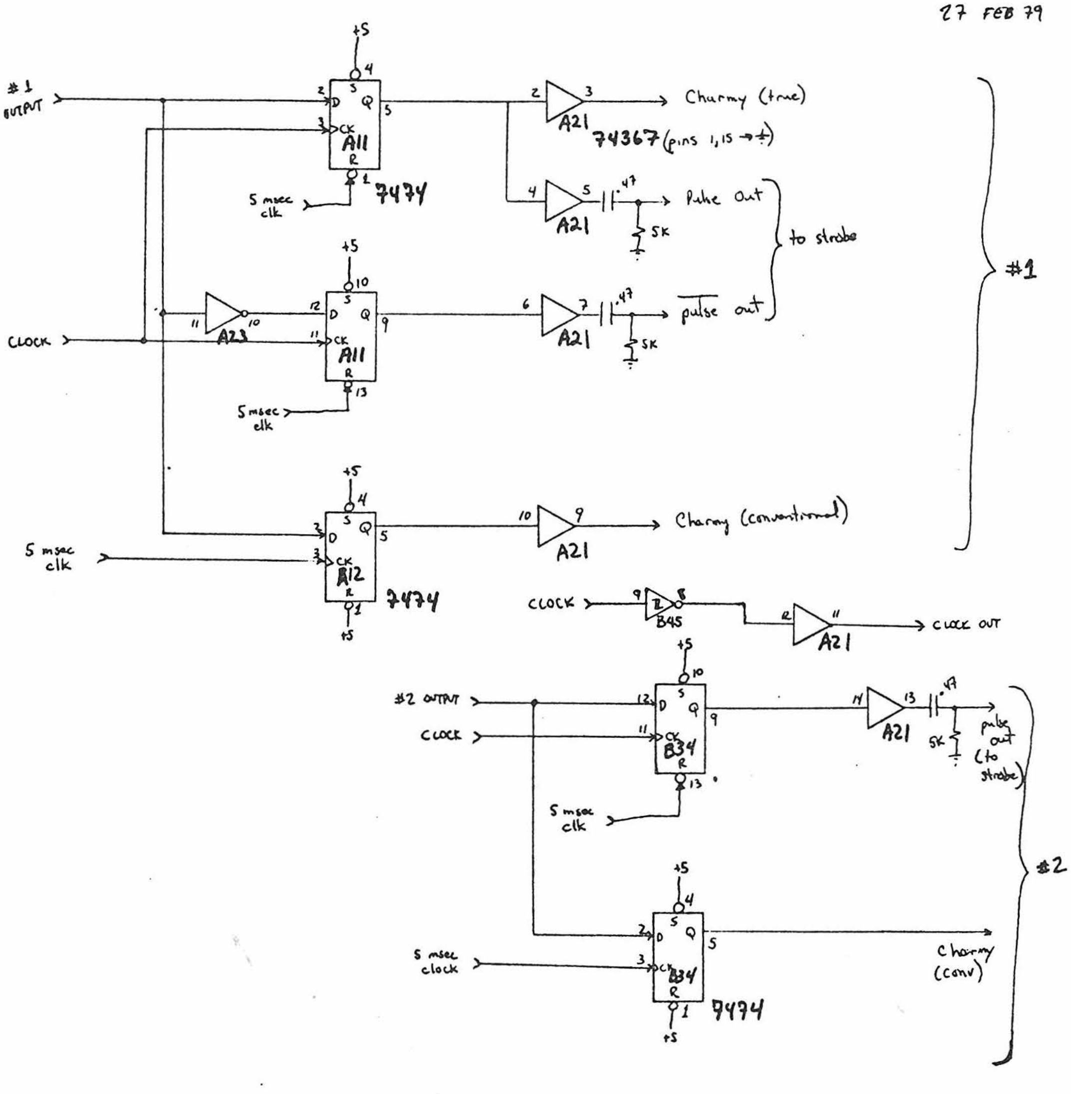

Figure 4A.1₃   The binary random sequence generator.

The output latches and buffers for driving Charmy and the strobe units (output #1; primary output: output #2; auxiliary).

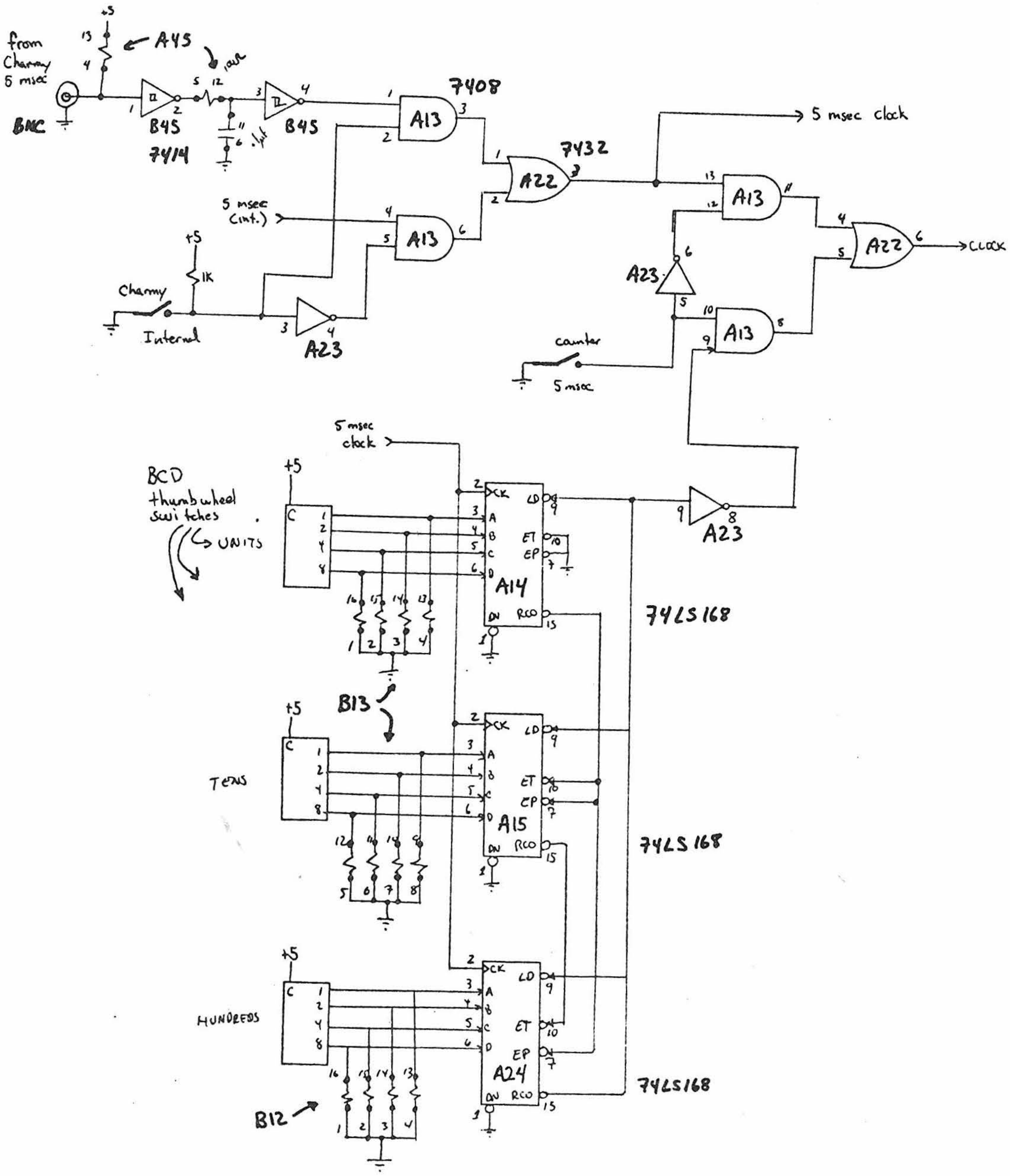
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Figure 4A.1₄ The binary random sequence generator.

Clock source determination (A13, A22).

Stimulus sample period logic (units of incoming clock periods, i.e. 5ms) (A14, A15, A24).

goggle electrode (Koblasz, 1978). This electrode is based on a pair of swimmers goggles which have a silver/silver chloride electrode mounted inside. To use the goggles, they are mounted facing down and filled with artificial tear fluid a mixture of salts and polyvinyl alcohol (PVA) is quite comfortable. The subject then places his eyes into the goggles and the tear fluid places the corners into electrical contact with the electrodes. They introduce (as do other types) noise from eye movements into the record, due mostly to the steady potential which is present on the cornea. This contamination can be reduced by good fixation, or by illuminating the other eye with an equal amount of steady light and measuring the ERG differentially between the eyes. The goggles have one (fairly important) advantage over the other types of electrodes, they provide a comfortable fluid interface which greatly reduces the need to blink which in turn provide very clean records of the ERG.

PREAMPLIFIER

The ERG signals are quite small, varying from about 5 microvolts (approximately) to perhaps 100 microvolts, thus they need to be amplified up to a level compatible with the data collection system and the kernel computer. Both of those pieces of equipment (as they are set up for these experiments) have input voltage ranges of ±5 volts, and therefore the gain of the preamplifier has set to 35,000 giving a range of about ±150 microvolts. The preamplifier used is a two-section device (figure 4A.2), with a front-end which is electrically isolated from the output end for the protection of the subject. The preamplifier also has various settings available for the low-pass and high-pass cutoffs for eliminating those portions of the signal which are not wanted. The

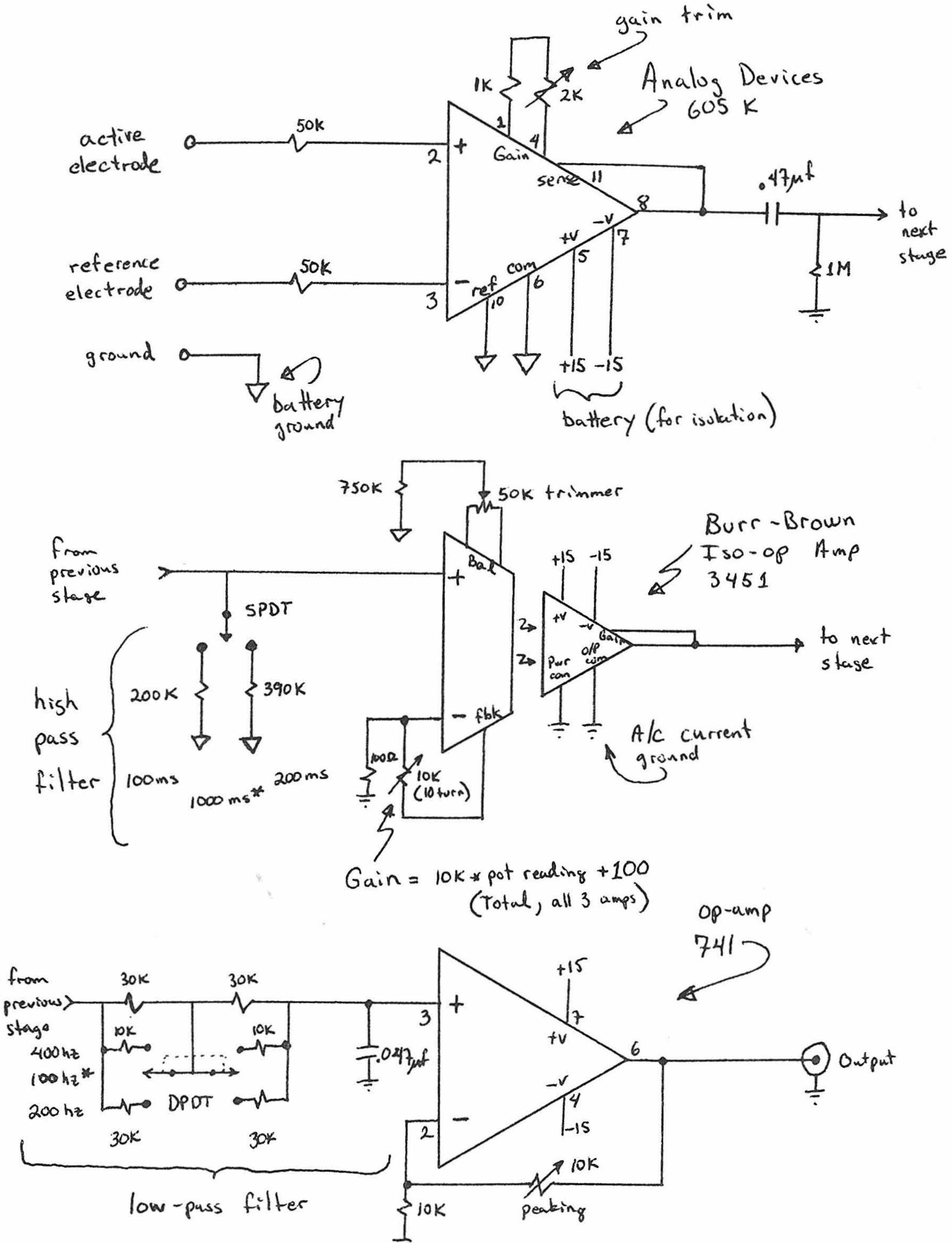


Figure 4A.2 The preamp.

The asterisks mark the normal settings of the filters. The gain was set at 35,000.

usual high-pass setting gives a time constant of about 1000 ms, and the low-pass setting cuts out those frequencies above 100 hertz to minimize aliasing problems with the 200 hertz sampling frequency.

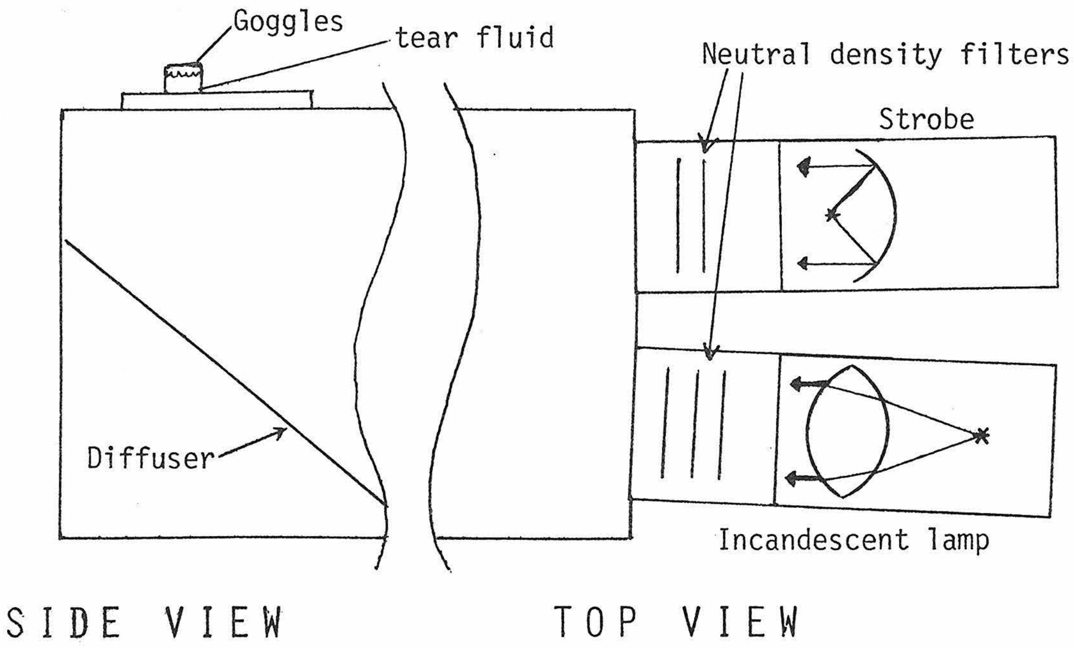


Figure 4A.3 The physical stimulus source.

The strobe lamp is used for the stimulus and the incandescent lamp is used for the background luminance.
The neutral density filters are used for gross luminance changes between runs.
The diffuser provides a large field view.

4b -- data gathering

The data presented in this thesis have come primarily from the research laboratory at Caltech and from the clinic at Doheny.

CLINIC (Doheny)

The data presented from Doheny are from normal subjects. The subject is dilated, anesthetized (corneally) and placed supine in a shielded room. The stimulus is delivered via a diffusing screen (for large field presentation) and a mirror placed in front of the subject. The subject then remains in the room while various parameters of the stimulus are varied in the gathering of the ERG. This procedure has been evolved to require as little cooperation from the subject as possible. This requirement is a necessity for success in a clinical environment as the patient population is generally nervous, not used to being subjects in this way, and often cannot see well enough to help (even to fixate a target).

The ERG signal is then amplified, fed to a kernel computer, (section 4d) and simultaneously recorded on a 4-channel FM cassette tape recorder. This tape is then brought to Caltech and transfered via a data collection system to 9 channel digital magnetic tape.

The cassettes are fed to the same data collection system that the laboratory data are transcribed with. This data collection system (Charmy) is a 128 channel multiplexer and digitizer which places the incoming data on standard 9-channel 800 b.p.i. digital magnetic tape ('mag tape'). These tapes are then readable by any of the large digital computers on campus.

LABORATORY (Caltech)

The procedure for gathering data in the laboratory was different because the subjects were well-trained, and thus were cooperative. The basic procedure is really quite simple, the data collection system is set up, the goggles are filled with tear fluid, the stimulus is presented and the response is recorded on mag tape by Charmy. The data were monitored with the kernel computer (section 4d).

For preliminary runs, and to debug the equipment, some experiments were performed without recording the data on mag tape, but instead only monitored with the kernel computer. Data from only one subject (RMC) used in the laboratory are presented in this thesis.

All of the data end up on mag tape from Charmy. These are then taken to the PDP 11/45 for further analysis. The system written by Dale Knudsen on the PDP 11/45 for the analysis of this type of data is called GAS (General Analysis System) and is used to compute kernels, average responses, auto-correlations, model responses, and just about anything else which might be needed.

To use GAS to calculate the kernels presented, one first transcribes the mag tapes from Charmy onto the DIVA disk, since the GAS system uses the disk as the primary data storage device. The prepared stimulus is then computed for the kernel calculation program by converting the raw stimulus to a +1, zero and -1 valued stimulus. This is done by combining the basic binary stimulus generated by the shift register with a timing sequence which contains the stimulus sample clock. Sections of the raw response (roughly 50 seconds each) are selected based on the lack of noise and the mean value of the response is then subtracted from the raw response generating the prepared response. The data from the clinic were then high-pass filtered at 5 hertz to remove some of the low frequency noise due to blinks and eye movements, this was not necessary in the data from the laboratory. This filtering was done with a digital filter which introduces no phase shifts.

The prepared stimulus and the prepared response are then fed to the program which calculates the kernels. These kernels may be hard-copied from the Tektronix graphic terminal on the PDP 11/45, or they may be copied to tape, taken to the IBM 370/3032 and plotted on a Calcomp plotter. A KS plotted on the Tektronix terminal has negative areas

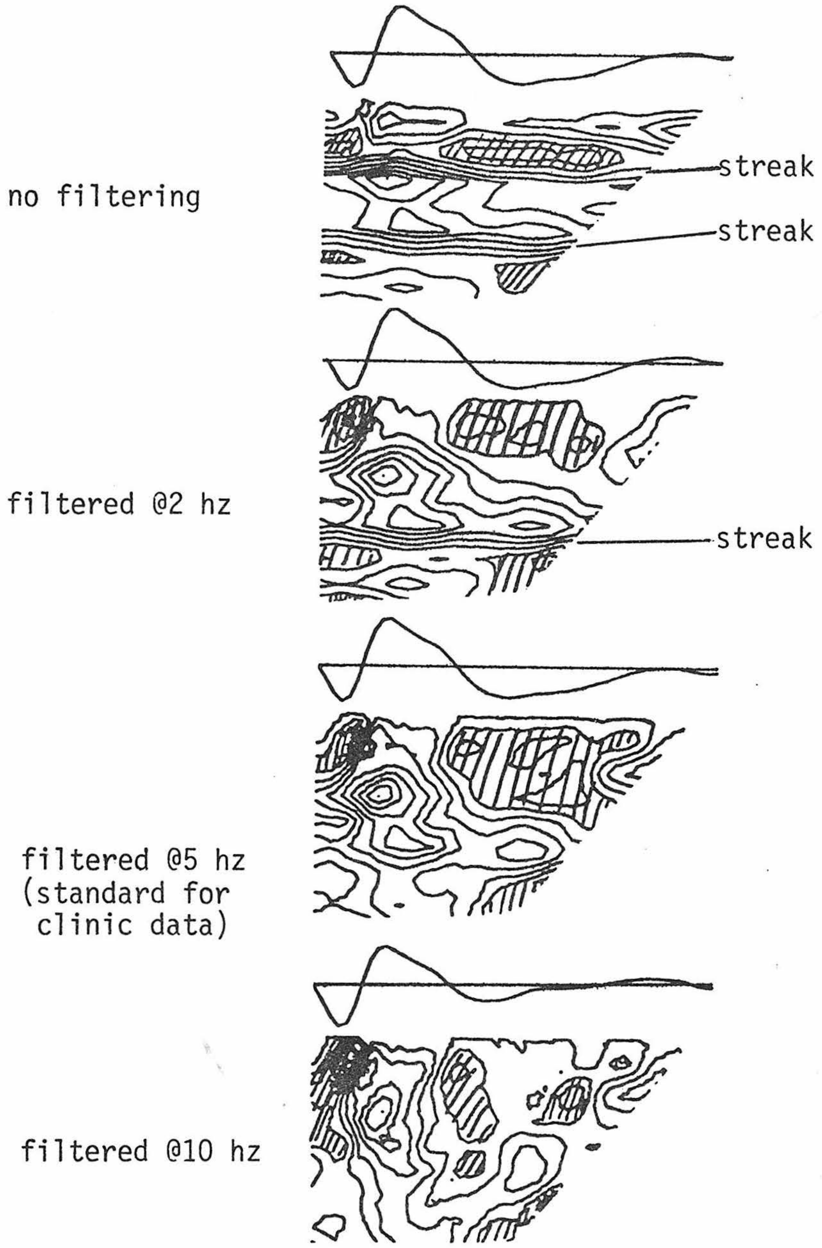


Figure 4C.1 Filtering and streaks.

Often when one plots the KS from noisy data, streaks appear through the plot, in a horizontal (for M2) direction. These streaks appear as diagonal streaks when plotted in M0.

These streaks are caused by adjacent cuts in the kernels which have different offsets (low frequency components).

The effects of the streaks can be minimized by high-pass filtering the data.

overlayed with dots, whereas a KS plotted on the Calcomp has positive areas overlayed with small '+' signs. These will be pointed out in the results as necessary.

4d -- kernel computer

When actually collecting data from an experiment using a random stimulus it is difficult to tell if the response which is being recorded is what you want, or if it is just noise. It was because of this problem and the desire for a faster turn-around that the idea for a real-time kernel computer originated.

In response to this idea, a kernel computer was designed and built, and subsequently simplified and placed in two clinics, at Doheny and at the University of California at Irvine. The kernel computer has been very helpful in collecting good data in the clinic and for monitoring the response in both the laboratory at Caltech and the clinics.

The kernel computer does the necessary cross-correlations between the stimulus and the response to provide the kernels as the experiment proceeds. It uses a ternary representation of the stimulus and an eight bit signed representation of the response. The internal memory for holding the kernels is 16 bits wide in the laboratory model, and 24 bits wide in the clinic models.

The KF is displayed on a CRT in a two dimensional format, just as it is here. The KS is displayed in MO (defined in section 3a) with the value of the KS at any point being represented by the intensity of the screen at that point.

The prototype computer and the subsequent computers which are in the clinics have some drawbacks which should be corrected in future models. Since the KS for the ERG is much more intelligible when displayed in M2 rather than MO, the KS display should be changed. The presentation should be in the form of cuts or contours rather than intensity. The intensity format is not satisfactory, and is difficult to hard-copy.

The kernel computer has been a great help in debugging equipment and keeping the experiments running in the laboratory, and has been the only way to collect white noise data from the clinics.

The actual circuitry of the kernel computer is extensive which precludes the inclusion of the schematics of the electronics. These schematics are on file in the electronics laboratory of the Bio-information systems department with the schematics of all of the electronic equipment developed here, On the following pages is a brief description of the operation of the kernel computer as it exists presently.

The internal operation of the kernel computer will be explained with the aid of figures 4D.1, 4D.2 and 4D.3. Figure 4D.1 is a block diagram of the arithmetic section of the kernel computer. This section receives the input response and stimulus values from the preamp and binary random sequence generator respectively. It then updates the representation of the kernel in memory.

The response is digitized into an 8 bit signed representation (7 bits + sign) and latched. The stimulus is received in its proper 2 bit format and stored in the stimulus memory. This memory behaves as a 64 (by 2 bit) first-in first-out memory.

The response and one (for the KF) or two (for the KS) values of the past stimulus values (from the stimulus memory) are multiplied and presented to the 16 bit adder. The past value of the kernel is also presented to the adder and the new value (the accumulated value) of the kernel is then stored back in the kernel memory.

To clear the present kernel from memory the 'clear' button is pressed which sets the memory to all zeroes.

Concurrently with the accumulation of the kernel the present value of the kernel is sent to the display section for real-time display of the present value of the kernel. The display section does not affect the arithmetic section in its operation.

Figure 4D.2 shows a block diagram of the address generation section of the kernel computer. The clock generator is a free-running oscillator which supplies the basic signals to the various sections to maintain synchrony. To start and stop the accumulation of the kernel, the compute/idle control is toggled. The clock is fed to the x-counter which supplies the address to the kernel memory and to the display section. This counter is a 6 bit counter with one extra count during which the KF is calculated (the rest of the cycles are used for the KS). The output of the x-counter is fed to the y-counter which operates in a similar manner to the x-counter. The output from the y-counter is fed to the offset counter which is added to the y-counter to provide the address to the stimulus memory. This is to make the stimulus memory behave as a recirculating first-in first-out memory.

Figure 4D.3 is a block diagram of the display section. There are two forms of output from the kernel computer; an oscilloscope (scope) and an x/y plotter. Either may be used to display the KF, but only the scope may display the KS or the output of the A/D converter.

The present value of the kernel from the arithmetic section arrives as a sixteen bit number which may be scaled by the scale selector and latched. The vertical DAC receives the A/D output when the input is being displayed; the kernel value when the KF is displayed; or the y-address when the KS is displayed.

The intensity (z-axis) DAC receives the kernel value when the KS is displayed, otherwise it is held constant. The horizontal DAC always displays the x-address.

The values of the x- and y-axis DAC's are used by the plotting logic to provide the output to the plotter. The KF is fed to the y-axis as the x-axis is scanned; at the end of the scan the pen is lifted and returned to the origin.

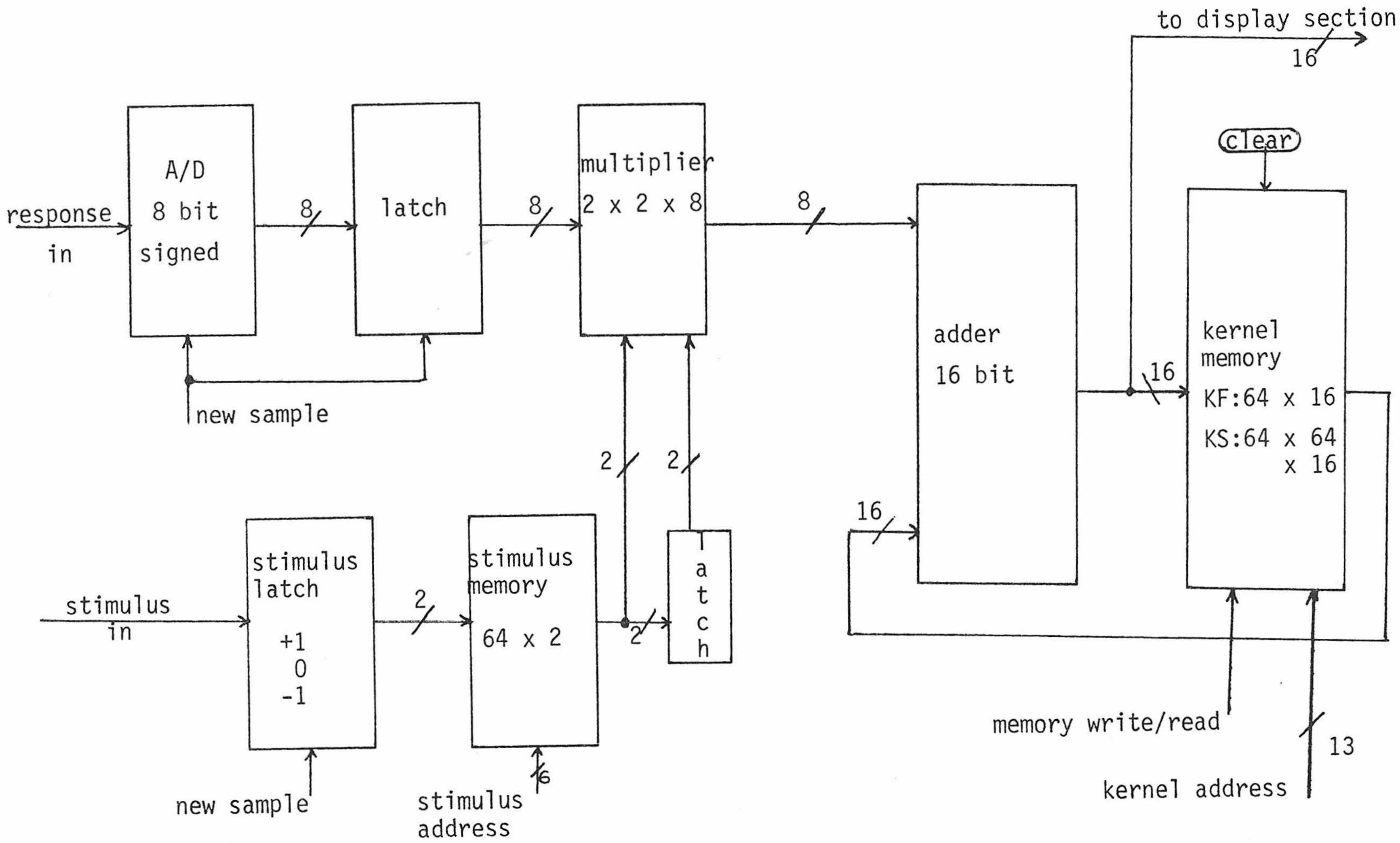


Figure 4D.1 Kernel computer -- Arithmetic section.

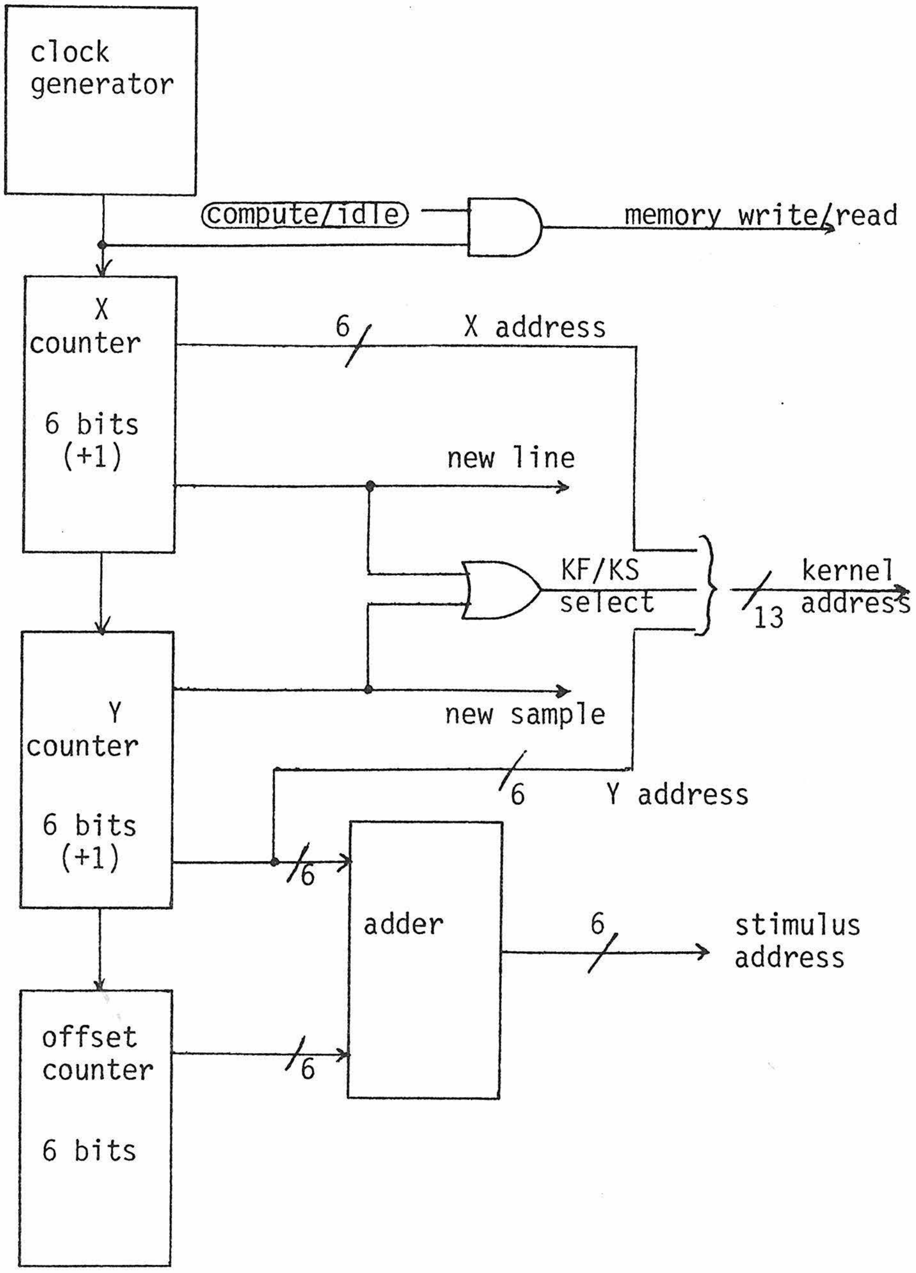


Figure 4D.2 Kernel computer -- Address generation section.

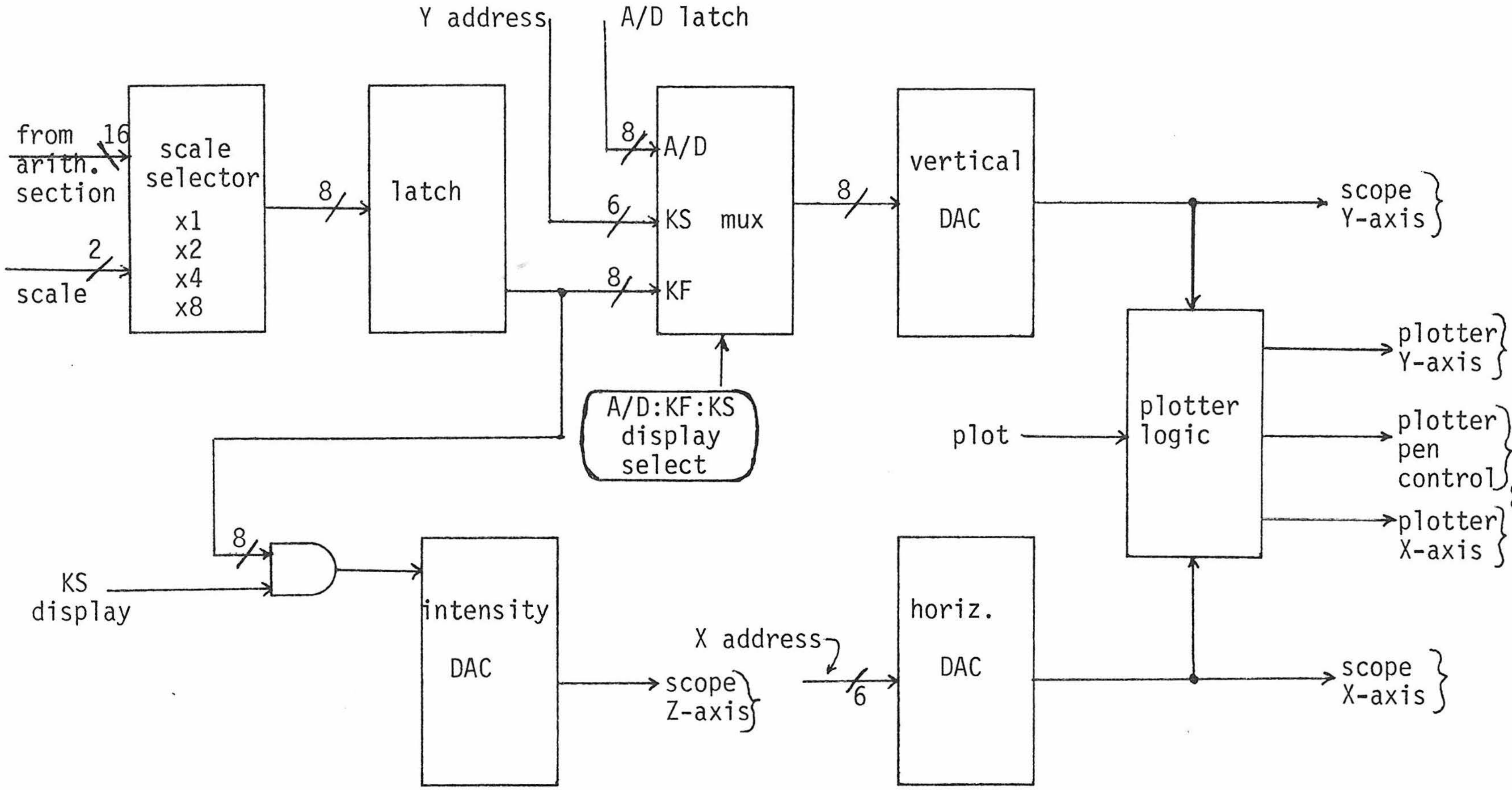


Figure 4D.3 Kernel computer -- Display section.

5) Results
(Adaptation in the ERG.)

5a -- linear adaptation

WIDE RANGE ADAPTATION

As mentioned in the introduction, one of the truly remarkable aspects of the visual system is its ability to adjust its sensitivity to a wide range of intensities. To investigate this behavior an intensity series is collected by inserting neutral density filters between the stimulus source and the subject. An intensity series is shown in figure 5A.1 with the KF's on the left, and a cut through each of the corresponding KS's (at 25 ms) on the right.

The relatively similar amplitudes of the KF's throughout the 200 to 1 change in stimulus intensity demonstrates the remarkable ability of the visual system to adapt. To see this effect more graphically, imagine scaling these kernels by the intensity of the stimulus, as in the white noise technique. If the brightest is left the same size the -2.6 log unit KF becomes about four feet high!

The choice of stimulus measurement techniques is well illustrated by this example. To represent the true sensitivity of the system use the white noise method of scaling. However, for practical reasons the conventional flash technique is probably best for this series.

Representative cuts through the KS's are plotted in the right column of figure 5A.1. Notice that although the amplitude of the KF is relatively constant, the amplitude of the cuts through the KS steadily increase through the series. (The vertical scale of the KS is one fourth that of the KF.) This means that the adaptation becomes more significant as the intensity of the stimulus increases.

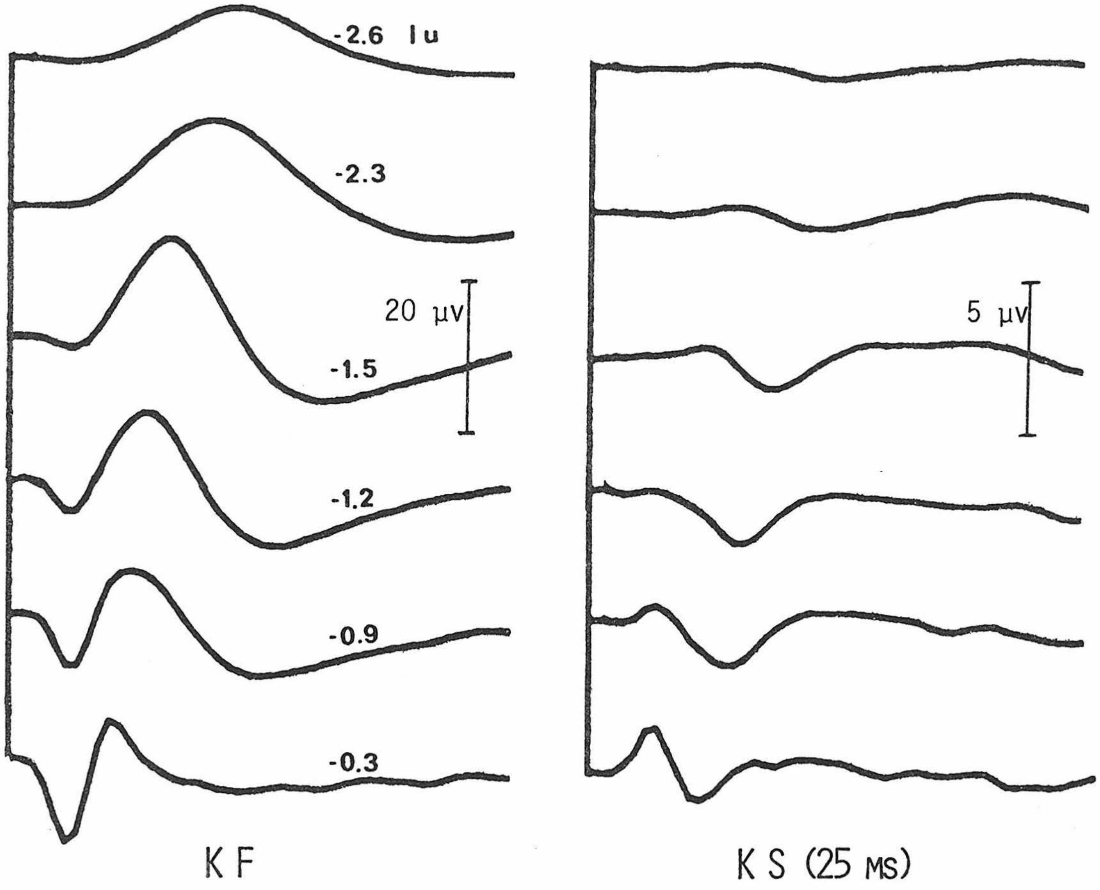


Figure 5A.1 Intensity series.

The series on the left is a group of first order kernels (KF) obtained with a white noise stimulus which varied in amplitude over a 200 to 1 (-2.6 log units to -0.3 lu) range.

The series on the left is a group of cuts through the corresponding second order kernels (KS). Notice that the first order kernels do not vary in size much (compared to the variation in size of the stimulus).

The next figure (5A.2) is a plot of the whole KS for the kernels of figure 5A.1. In the previous figure one of the notable features was the compression of the response toward the origin as the light intensity increased. This compression may also be seen in the horizontal axis of the KS. The dynamics of the adaptation are represented by the way that the KS equivalent of the a- and b-waves dies out in the 'time between stimuli' axis. Notice that initially, at lower intensities, the b-wave outlasts and is larger than the a-wave in the KS, but as the stimulus becomes brighter, the situation reverses.

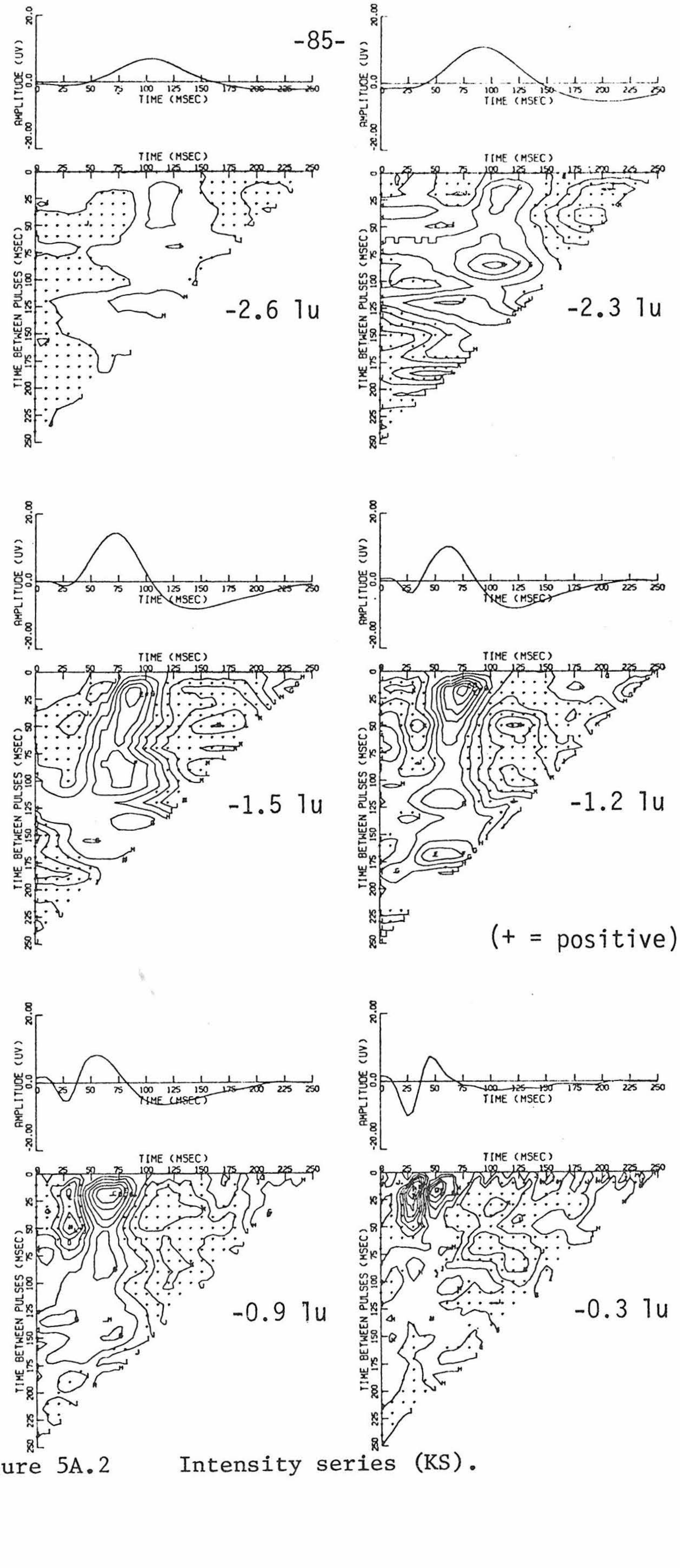


Figure 5A.2 Intensity series (KS).

WHITE NOISE VS. FLASH KERNELS

When white noise kernels are compared to flash kernels (average flash responses), they are almost invariably different. Thus it seems as though white noise is a different type of stimulus which is not at all comparable to flashes. This is not so. Actually, most of the difference is between photopic and scotopic responses.

To demonstrate this, two experiments were performed. All of the flashes for both experiments were white and had the same amplitude. In the first experiment a kernel was obtained from a rapid random flash stimulus with a sample rate of 15 ms, then successive kernels were obtained with a 30 ms stimulus sample rate, 60 ms, 120 ms and finally 240 ms. The average flash rate in the last run is only about 2 hz, which is well within the range of flashes. As the flashes became less frequent, the average intensity of the stimulus decreased, this was compensated for by measuring the average stimulus intensity with an integrating telephotometer and adding in sufficient steady light to bring the total luminance back up to the level (80 candelas) at the beginning of the experiment.

This experiment is illustrated in figure 5A.3. As can be seen, the shape and size of the a- and b-waves are roughly constant throughout the series, thus showing that for these waves at least, the system reacts to white noise as it does for widely spaced flashes. The residual slow negative wave at about 125 ms does not remain the same. This r-wave, as we call it, is not normally noticed in the ERG. More will be said about it shortly.

The second experiment was a continuation of the first, the same

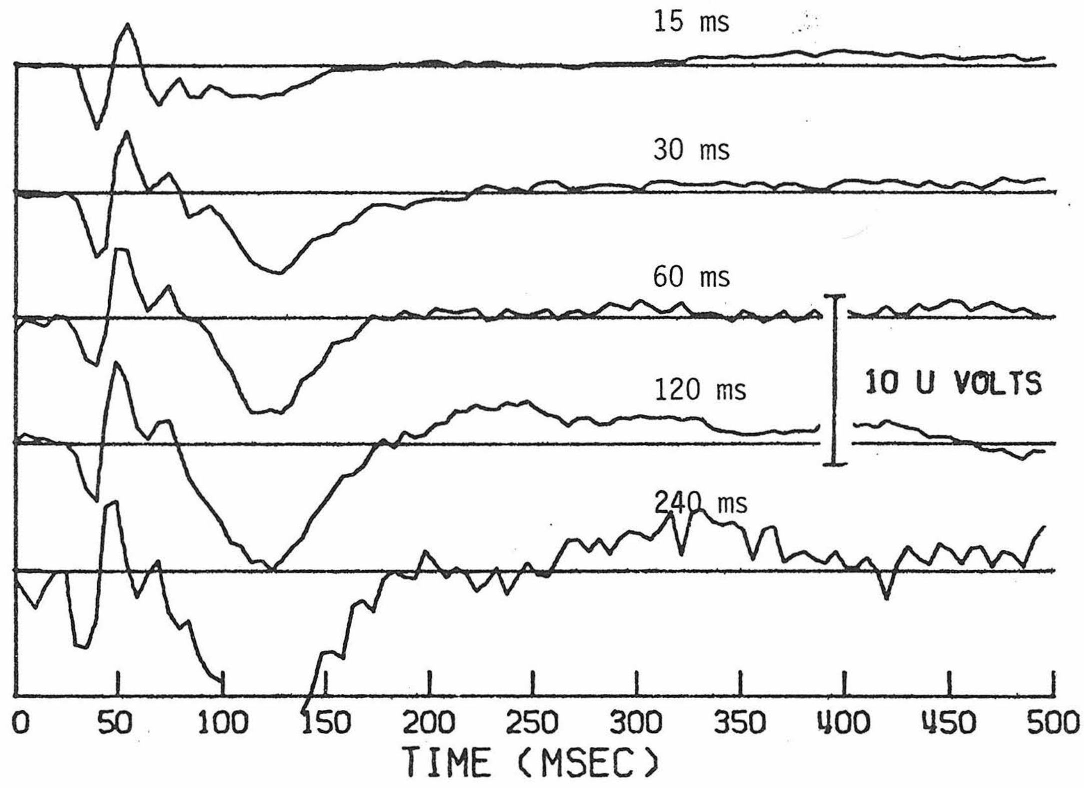


Figure 5A.3 Stimulus sample period series.

For this series each flash is the same size, the difference is in the average flash rate. The average luminance is also kept the same for each run by adding a background to match the luminance lost by reducing the flash rate.

240 ms stimulus sample rate and the same flash intensity, but in this series the steady light added to the stimulus was reduced in three steps until the flashes were presented in darkness. The changes in the ERG due to this change in the stimulus account for nearly all of the apparent differences between the white noise and flash kernels (figure 5A.4). A sample of the raw data from this experiment is shown in figure 5A.5, notice that when the background is removed from the stimulus, the waveforms can be seen without any signal averaging.

Thus, the differences between white noise and flashes (even when the same size flashes are used) is due mostly to the higher effective background level caused by the many rapid flashes in the white noise stimulus.

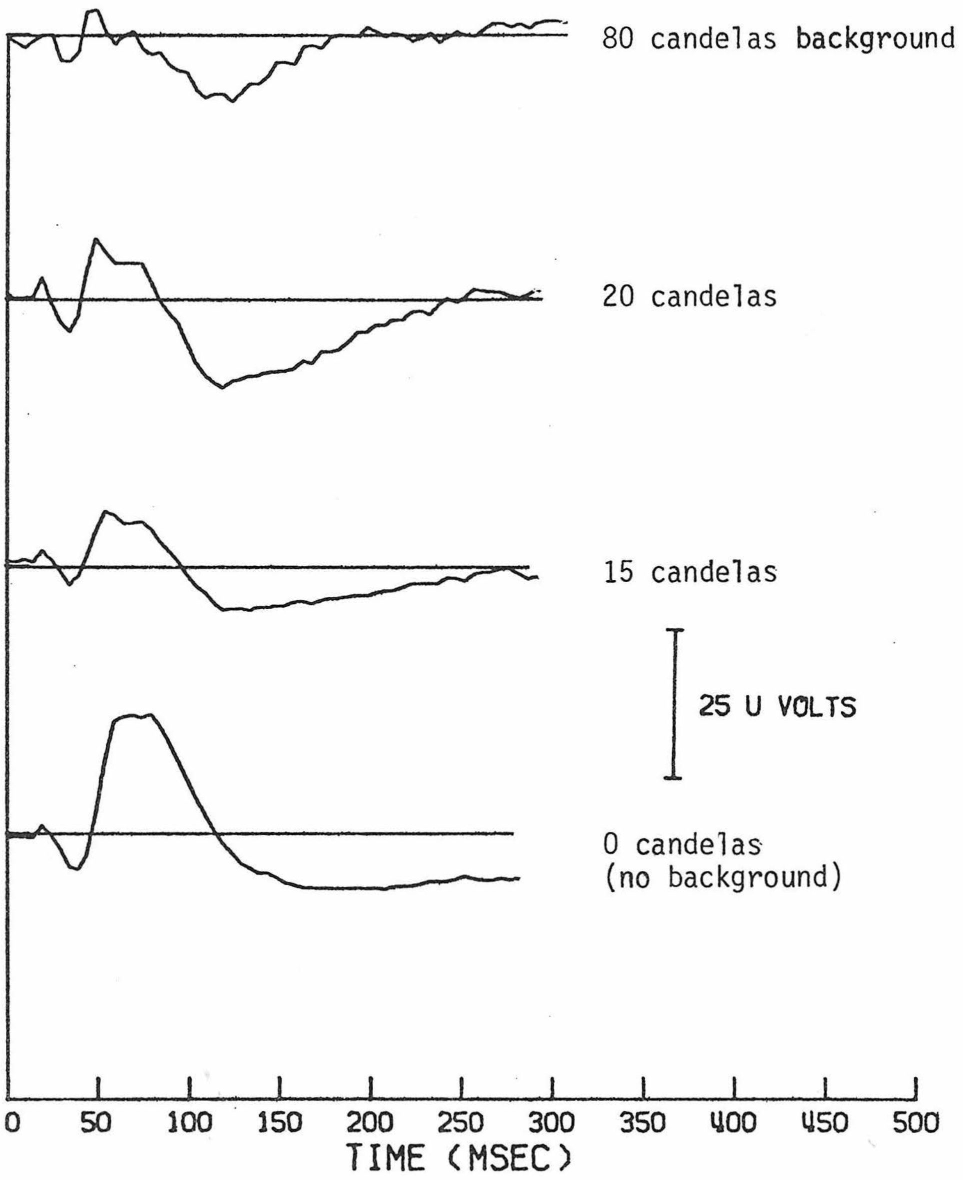


Figure 5A.4 Background level series.

The flash size for each of the kernels in this figure is the same as that for figure 5A.3. The stimulus sample period for the kernels in this figure is 240 ms. The difference between these kernels is in the amount of background light added. The top KF is the same as the bottom KF in figure 5A.3, the bottom KF is from flashes on darkness, the other two are intermediate values.

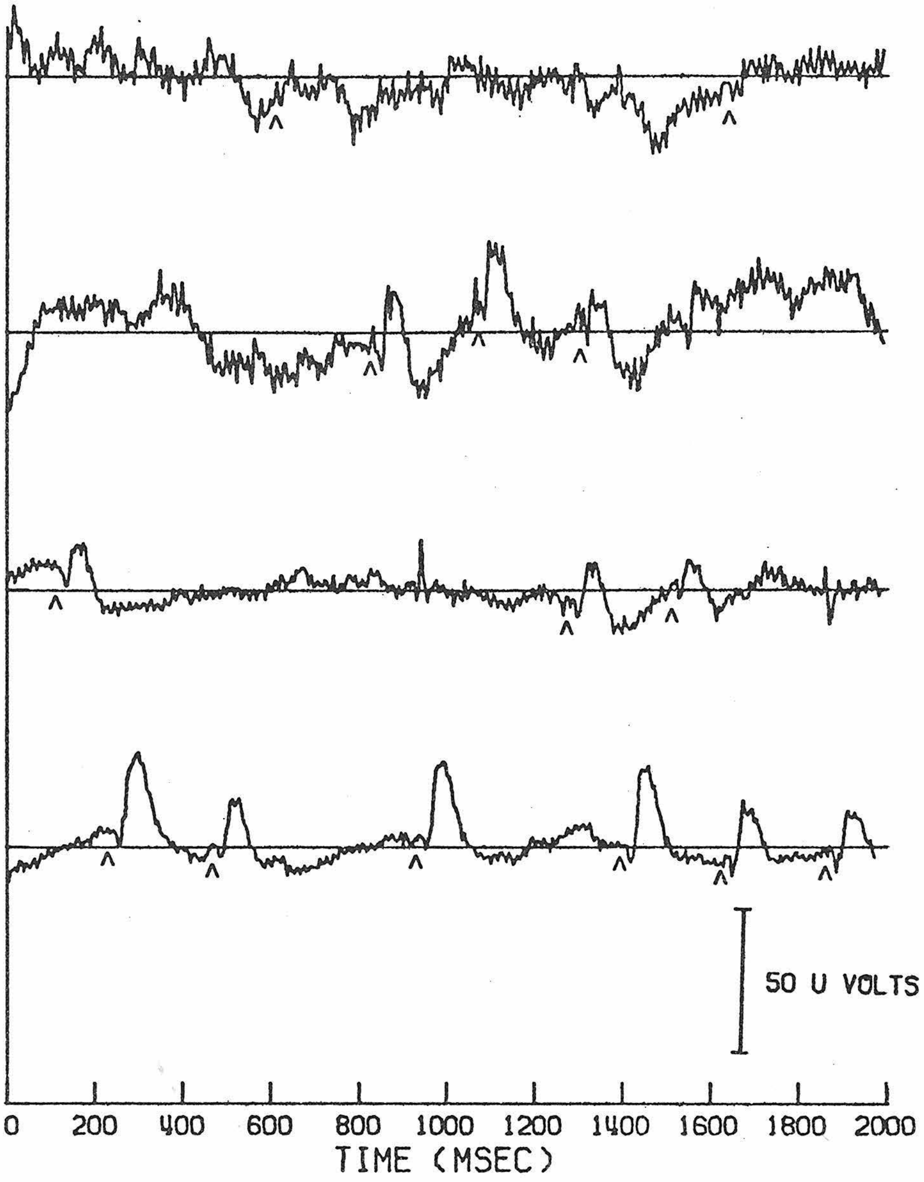


Figure 5A.5 Raw data from background series.

The ^ shows where a flash occurred.

BACKGROUND ADAPTATION AND THE R-WAVE

In the last experiment there was a residual wave which did not behave in the same manner as the a- and b-waves. The origins of this wave are unknown, but some observations which we have made suggest a possibility. Basically the possibility which we have explored is adaptation (suppression) of the sustained response of the ERG to the background (or effective background in the case of white noise). The ERG is known to have components (probably at least two, from the receptors and the b-wave source) which give a sustained response to a constant light (Brown, 1968).

Since we have shown adaptation dynamics in the ERG that are comparable to dynamics of the response, it does not seem unlikely that adaptation might have some effects upon the ERG which have similar appearances to 'real' waves. We are proposing that the adaptation which acts to suppress the response to a flash might also affect the sustained response.

There are two indirect reasons for believing that adaptation of the background response is responsible for the r-wave. The first is illustrated in figure 5A.6, a KF and a KS (shown as cuts) for a representative ERG. The relevant aspect of this figure is that there are waves in the KS cuts which correspond to the a-wave, the b-wave and to the portion of the LRP which (presumably) occurs after the b-wave. However, there is no wave in the KS which corresponds to the r-wave! There is something fundamentally different about the r-wave when compared to the a- and b-waves, it does not undergo suppression when preceded by a flash (at least as seen in the KS).

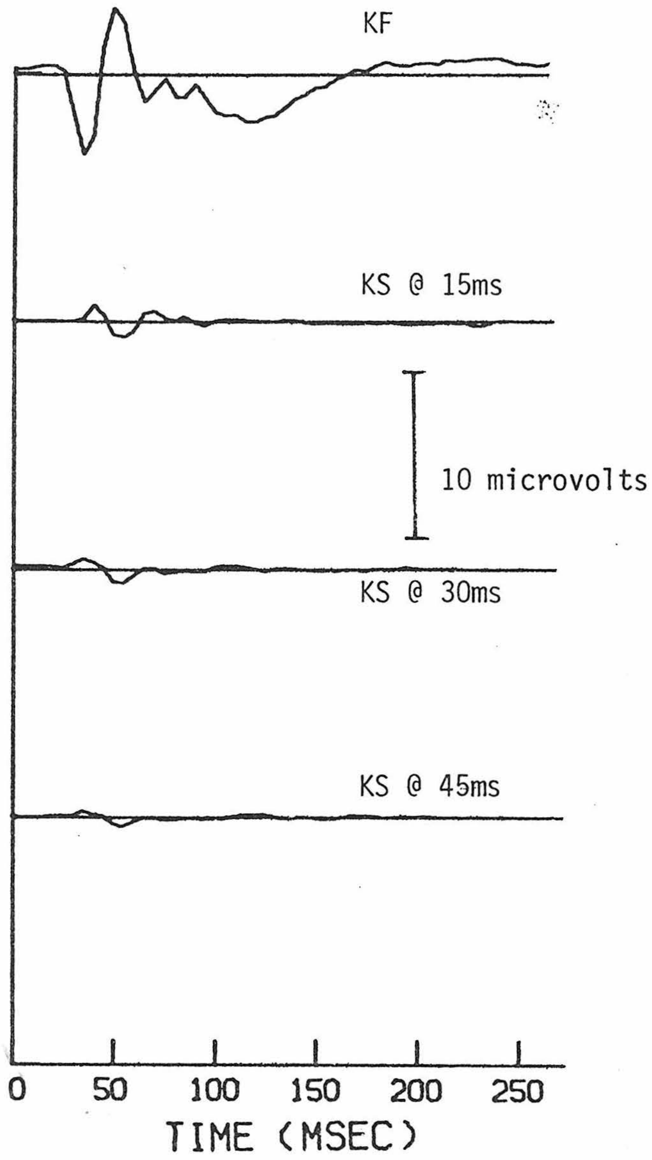


Figure 5A.6 KF and KS with white light.

The KF and three cuts through the KS for white light. Notice that although the KS has waves corresponding to the a- and b-waves, there is no wave in the KS corresponding to the r-wave in the KF.

The second reason is that the model of Troelstra and Schweitzer predicts a similar phenomenon. They used a shape for the b-wave which was monophasic and totally positive. When they used their model to predict the response to a flash presented on a constant light, rather than in darkness, they found a negative afterpotential following the b-wave. This is because their model predicted the sustained response and the transient suppression of that response by a flash.

To make the situation more involved, see figure 5A.7, which compares the kernel of figure 5A.6 with that obtained from a red colored stimulus. The a- and b-waves are very similar, but the r-wave is inverted. The corresponding KS (figure 5A.8) shows that there is again no wave in the KS analogous to the r-wave in the KF.

The different dynamics of the a- and b-wave rapid adaptation seen in the two KS's (white: figure 5A.6, red: figure 5A.8) offer an explanation for the inverted r-wave. The white KS has an emphasis on the b-wave, whereas the red KS has more of an emphasis on the a-wave. Therefore, the white KS should emphasize the adaptation of the b-wave and show a negative afterpotential, and the red KS should emphasize the adaptation of the a-wave and show a positive afterpotential. (Suppression of the negative sustained a-wave will be expressed in the KF as a positive potential.)

The linearity of the r-wave (it does not appear in the KS) is explained because it is not actually a component, but the effects of the adaptation expressed in the KS. Since the KS expresses linear adaptation (Section 3A), any effect due directly to adaptation will also be linear and not appear in the KS.

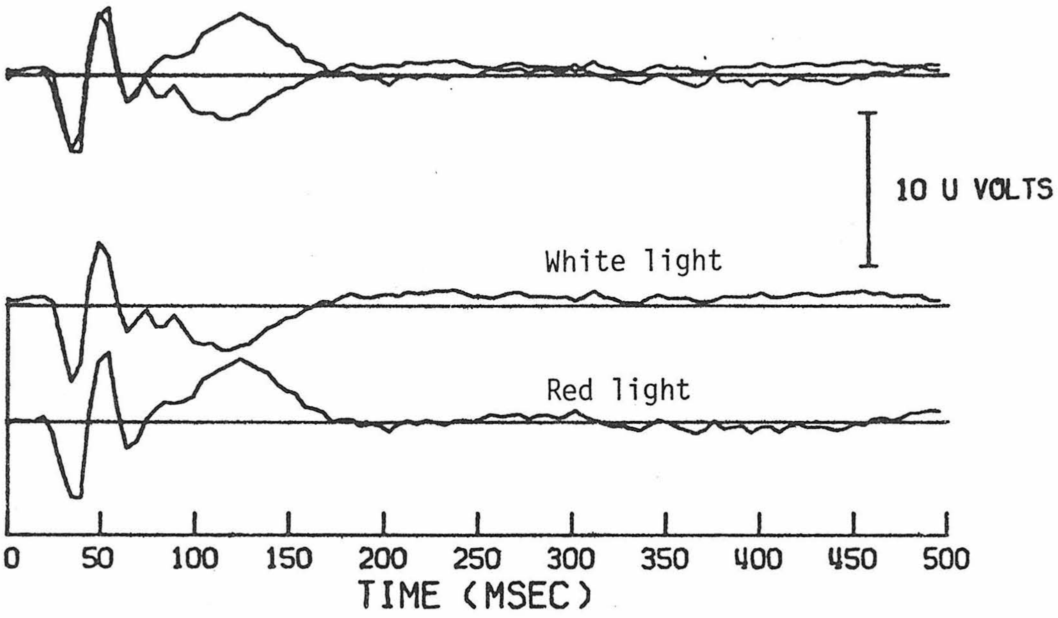


Figure 5A.7 The r-wave in the KF.

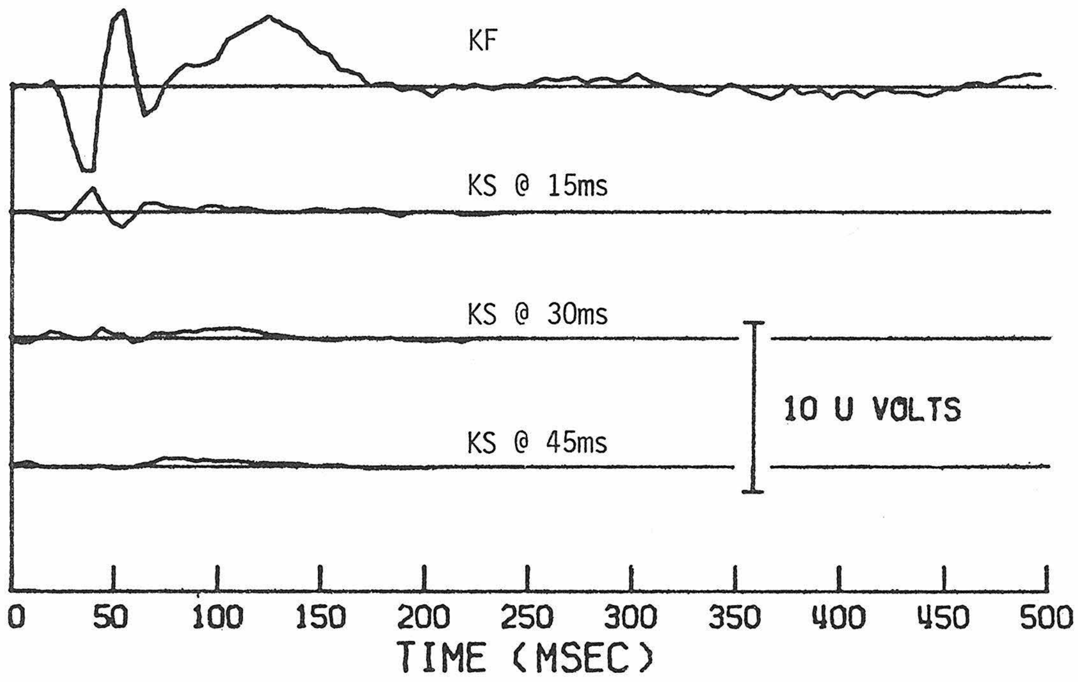


Figure 5A.8 The KF and KS with red light.

These data do not prove nor even present direct evidence to support this suggestion of background adaptation, but they do provide a plausible mechanism to account for the presence of this unusual wave in the ERG.

5b -- suppression-recovery and the KT

In the last section, linear adaptation was explored and the KS was shown to represent this phenomenon in a manner compatible with the intuitions which most are familiar with. The basic observation was that darkness increased the sensitivity of the system and that brightness decreased the sensitivity. There is evidence that the amount of suppression which is invoked by a conditioning flash is not simply a function of the size of the flash, as assumed by the KS, but that the amount of adaptation (suppression) is also related to more than the conditioning stimulus alone. The most obvious experiment which shows this effect is the response to the beginning of an applied flash train.

The response of the cat ERG to the onset of a flash train of various intensities is shown in figure 5B.1 (from Arden, Granit, and Ponte, 1960). The effect is best seen in the run at 119,000 lux. The response to the first flash is a given size; this can be predicted by the KF. The response to the second flash is smaller than the first; this is linear adaptation and can be explained by the KS. But, the response to the third flash is larger than the second; this cannot be explained by the KF and the KS -- they would predict that the third response would be smaller than the second and so forth. Thus, some other explanation is needed. The recovery of the system from the suppression of the response to the second flash has been termed suppression-recovery.

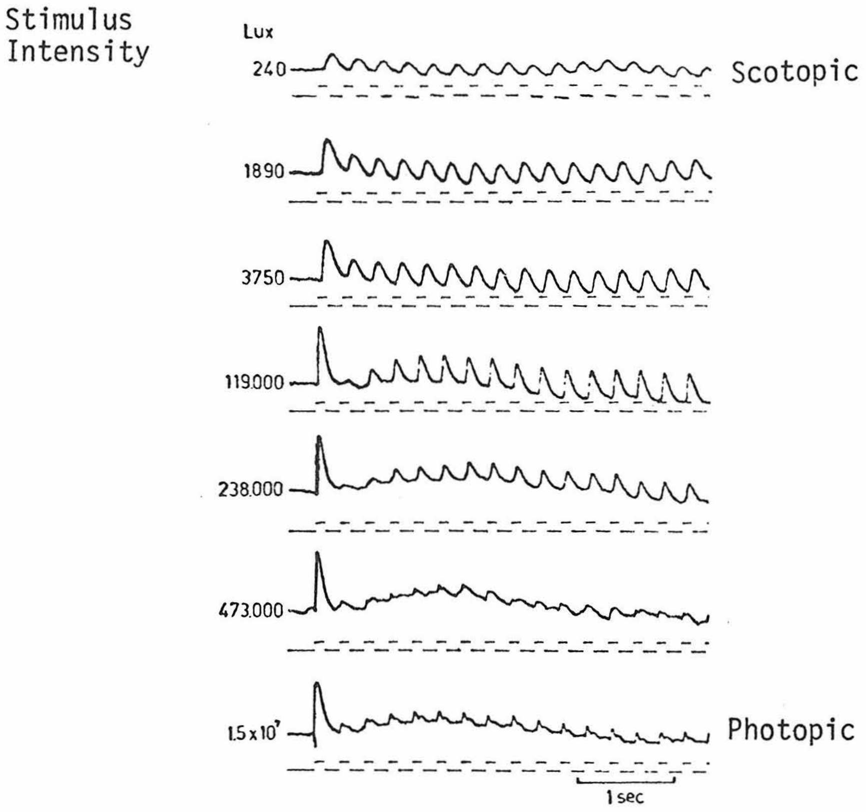


Figure 5B.1 Suppression-recovery.

(from Arden, Granit and Ponte; 1960)

THE KT

The extension of the KF and the KS which may account for this effect is the third order kernel (the KT). The KT represents the effect of a third flash (a second conditioning flash, section 3b) on the KS. The KF and the corresponding KS and KT from the -0.9 log unit response of figure 5A.1 is shown in figure 5B.2. The KF and KS are displayed in the usual format, but the KT being in its entirety a four-dimensional entity, cannot be displayed on a sheet of paper. Thus, two cuts through the KT are displayed. These cuts are labeled A and B and have different interpretations since they are cuts in different directions through the KT.

The relationship between these two cuts and the KS is shown in figure 5B.3. The cut labeled B corresponds to the change in the whole KS due to the presence of a second conditioning flash 25 msec before the first conditioning flash. The KT cut labeled A corresponds to the KS cut at 25 msec (displayed to the right of the KS). It shows the effect of a second conditioning flash which occurs earlier than a pair of flashes 25 msec apart.

The B cut is interpreted by overlaying it on the KS -- for this particular cut it is roughly the same as the KS, but with the polarity reversed. This is similar to the relationship of KS cuts and the KF. The A cut is interpreted by comparing it to the KS cut at 25 msec.

SUPPRESSION-RECOVERY

That the effects of the KT can account for suppression-recovery can be seen in figure 5B.4. The top of the figure shows how the different kernels are added together to form the system's response to different

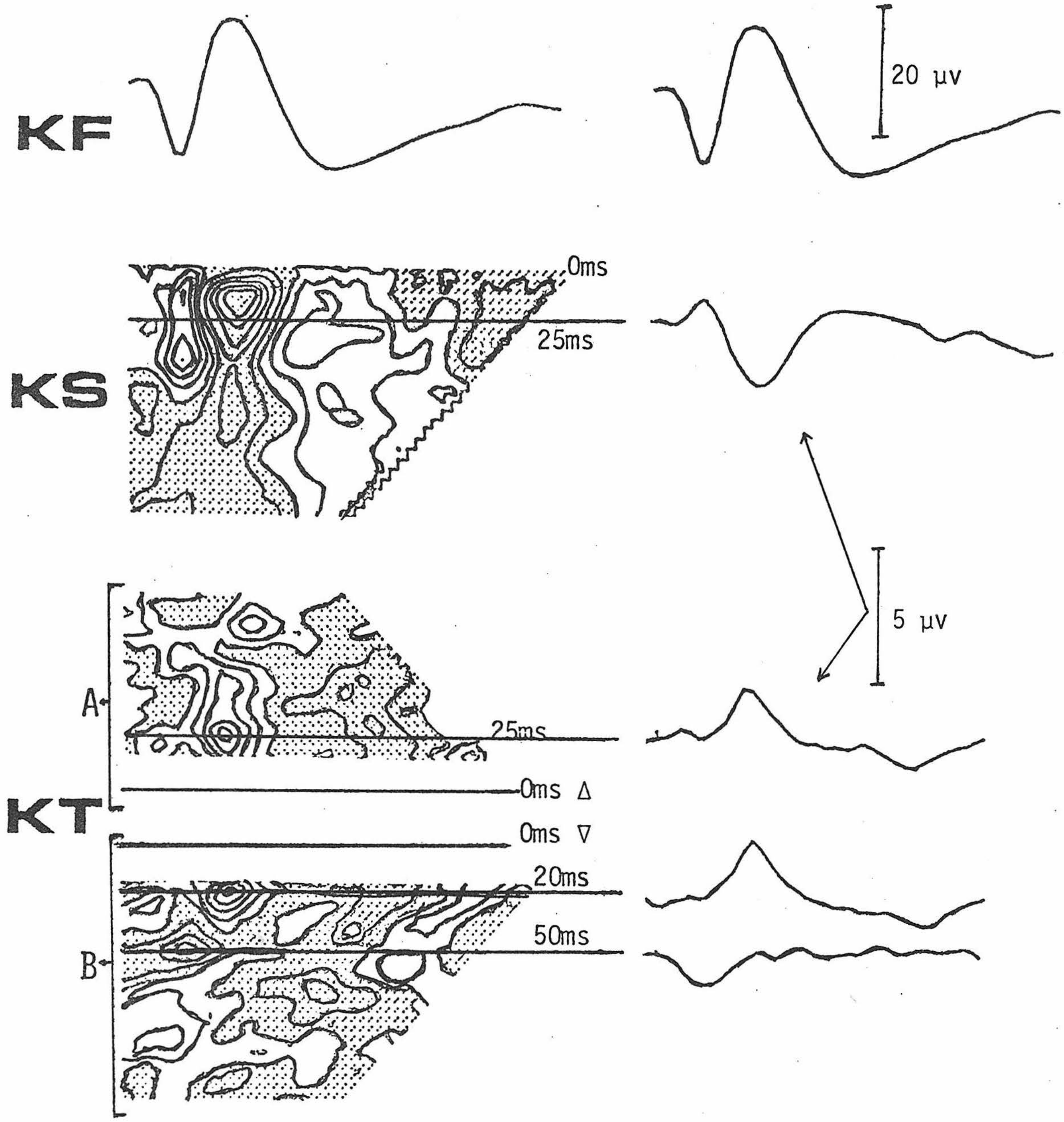


Figure 5B.2 The KT.

The stimulus sample rate for these kernels was 15ms. This makes the kernels undefined within 15ms of any diagonal. The second order kernel main diagonal has been set to zero. The undefined areas of the third order kernel have been left blank for simplicity of display.

A) Cut through the KT @ 25ms between the second conditioning flash and the test flash.

B) Cut through the KT @ 25ms between the first and the second conditioning flashes.

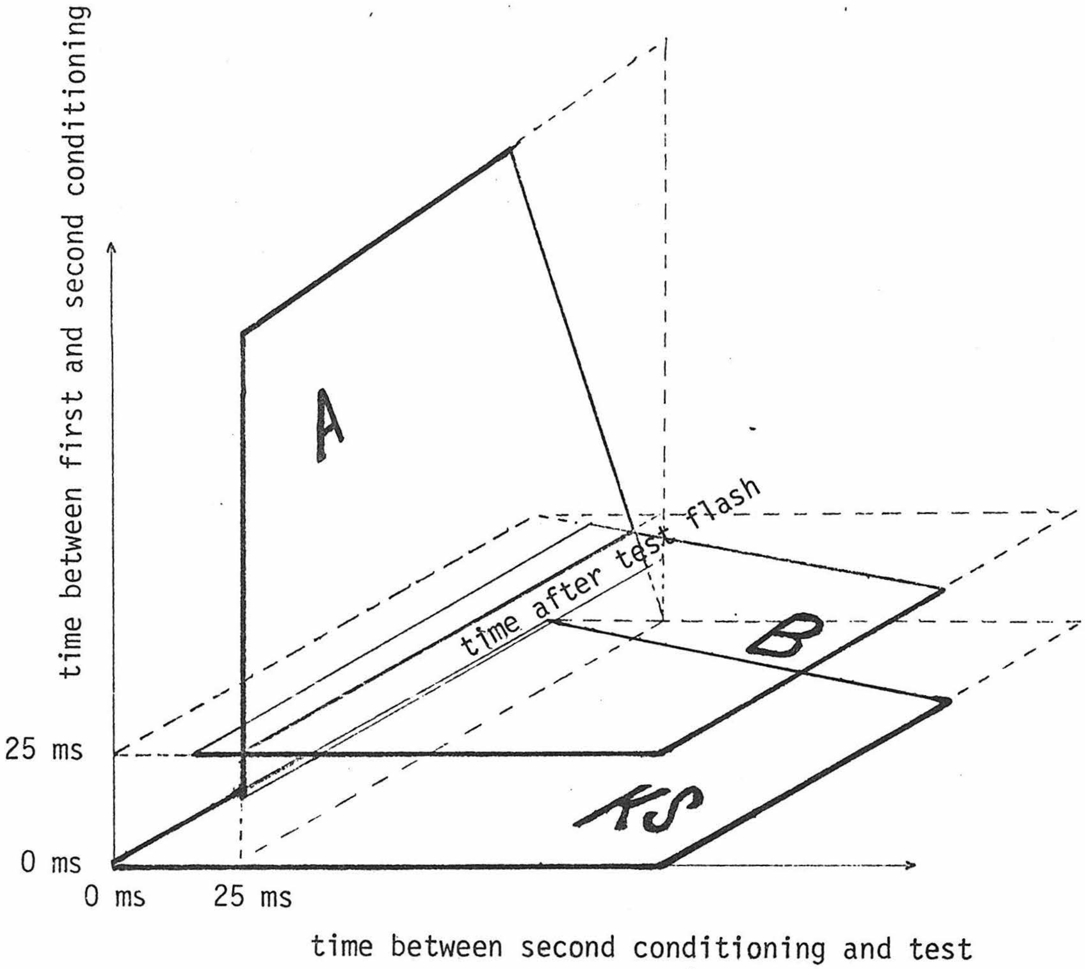
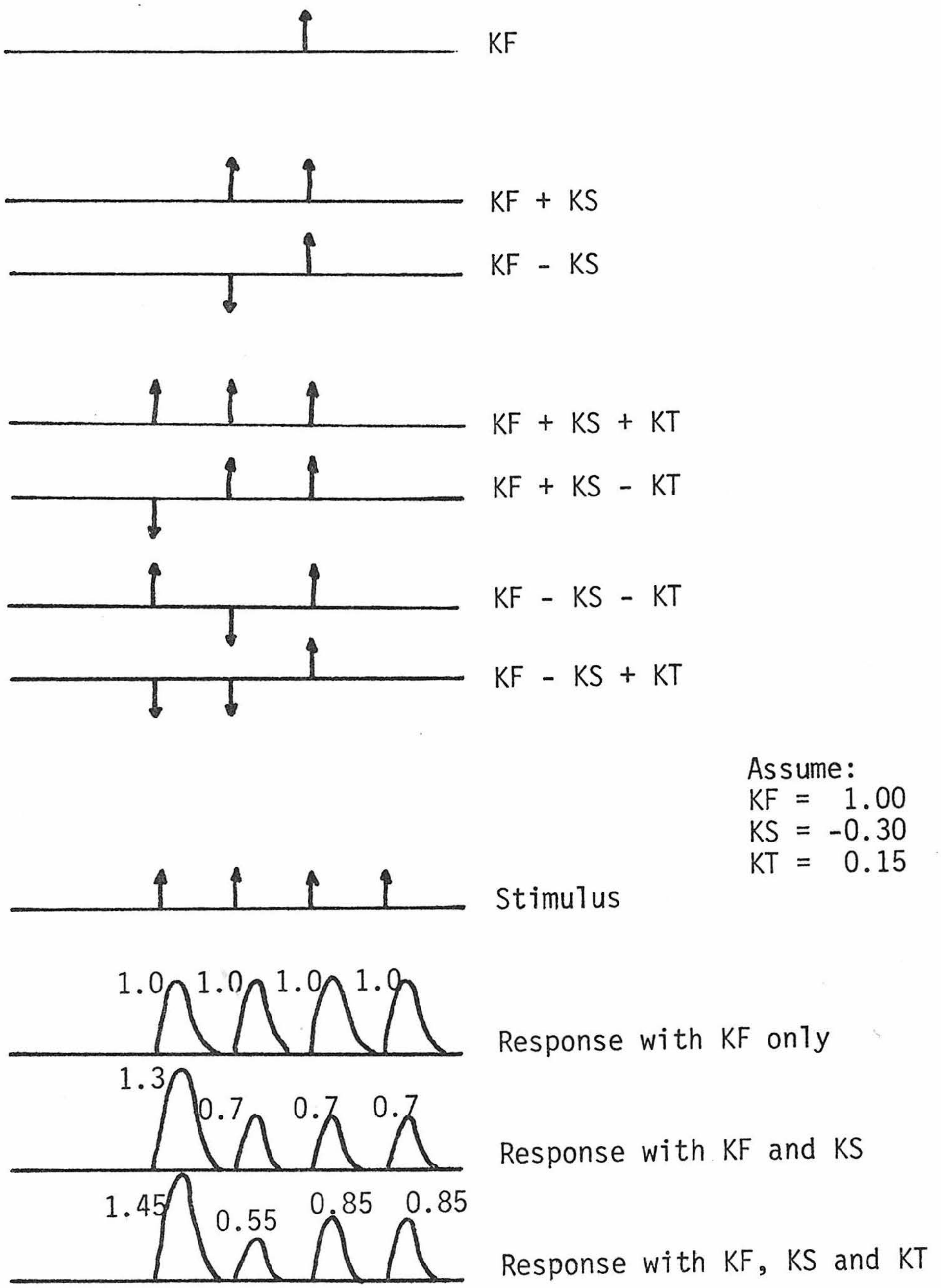


Figure 5B.3   Interpreting the KT.



Assume:
KF = 1.00
KS = -0.30
KT = 0.15

Figure 5B.4 Suppression-recovery and the KT.

patterns of flashes. An up-arrow means a flash and a down-arrow means a 'negative' flash, i.e. a short period of darkness.

The response shown is a stylized b-wave short enough to avoid response overlap, rather than the actual ERG, for simplicity. The relative amplitudes (rather than the shapes) of the responses to the flashes is the effect being illustrated. The sizes of the KF, KS and KT are 1.0, -0.3 and 0.15 respectively which are reasonably similar to those obtained from kernels from a photopic stimulus. A definite suppression-recovery effect is evident. In figure 5B.4, the adaptation present was assumed to last until the next flash, but not to last for two flashes. If this assumption is removed and the true dynamics of each of the kernels were used, the recovery period would last longer than one flash and the whole effect would be very similar to those of figure 5B.1.

The KT for the ERG has the proper configuration and polarity to account for the suppression-recovery effect. It has advantages in the study of this effect analogous to those of the KS in linear adaptation. The KT, for the photopic response used, has a magnitude which is not at all negligible; it is about half the size of the KS.

5c -- component identification

In an experiment at Doheny, a number of monkeys were given vitreous injections of blood in a study of vitreal hemorrhages and their effects on retinal degeneration and eventual detachment. During the experiment the conventional average flash response was obtained along with rapid random flash kernels.

These data are not presented here for the purpose of making any statement about the physiology of vitreal hemorrhages, but rather because of a secondary use of the KS. A brief summary of the physiological effects will be given for perspective. The effects were two-fold -- 1) the blood is nearly opaque initially and absorbs most of the light entering the eye before it reaches the retina, and 2) the iron of the hemoglobin in the blood poisons the retina and slowly (over a period of weeks) changes the shape of the ERG. It is the use of the KS as an aid to component identification in the abnormal ERGs collected during this study that will be discussed.

To obtain a norm, the kernels for the unaffected eye of the monkey are presented in figure 5C.1 and 5C.2. Notice, as for the human ERG, that there is an 'r-wave' in the -1 log unit response. That this is not a second b-wave, but instead probably adaptation of the LRP (as before, section 5a - background adaptation) can be seen by noticing that there is no component in the KS corresponding to the second 'b-wave'. The scotopic ERG is probably too weak to elicit the 'r-wave' and the bright photopic kernel has an 'r-wave', but is cancelled instead by the trailing edge of the LRP.

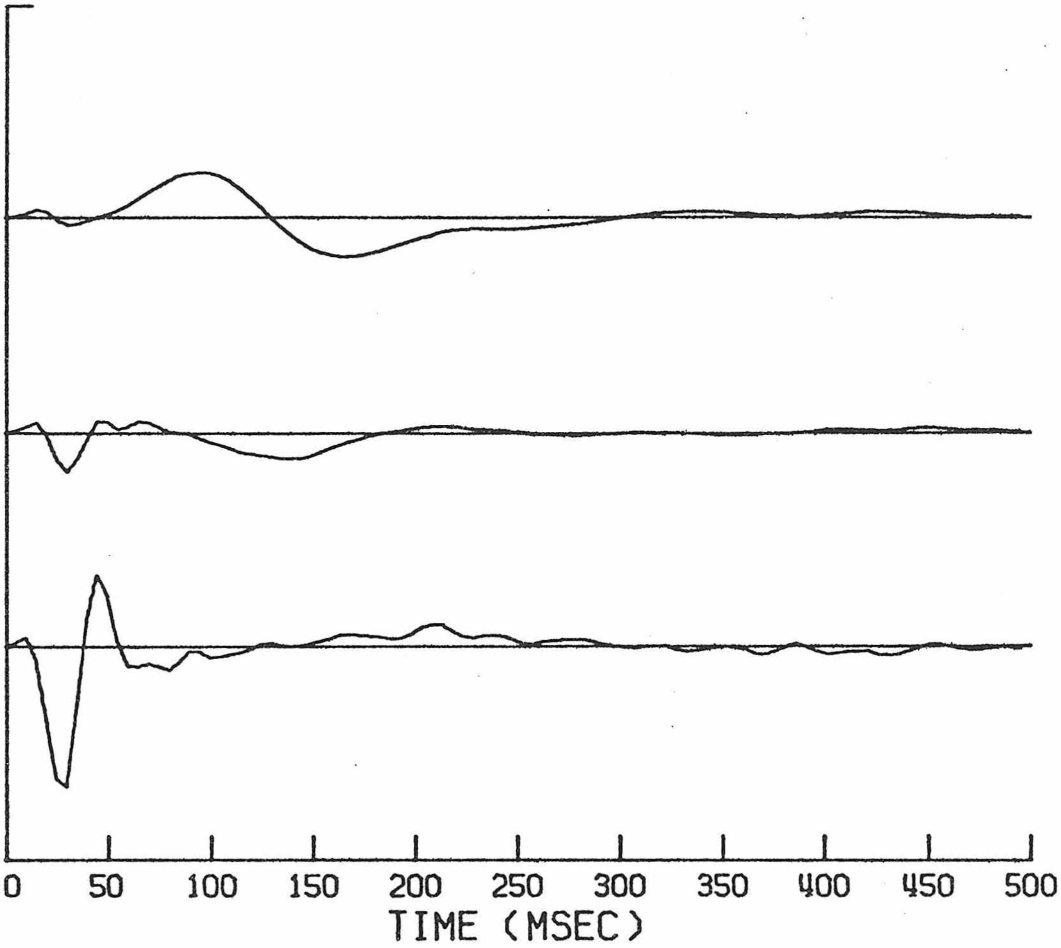
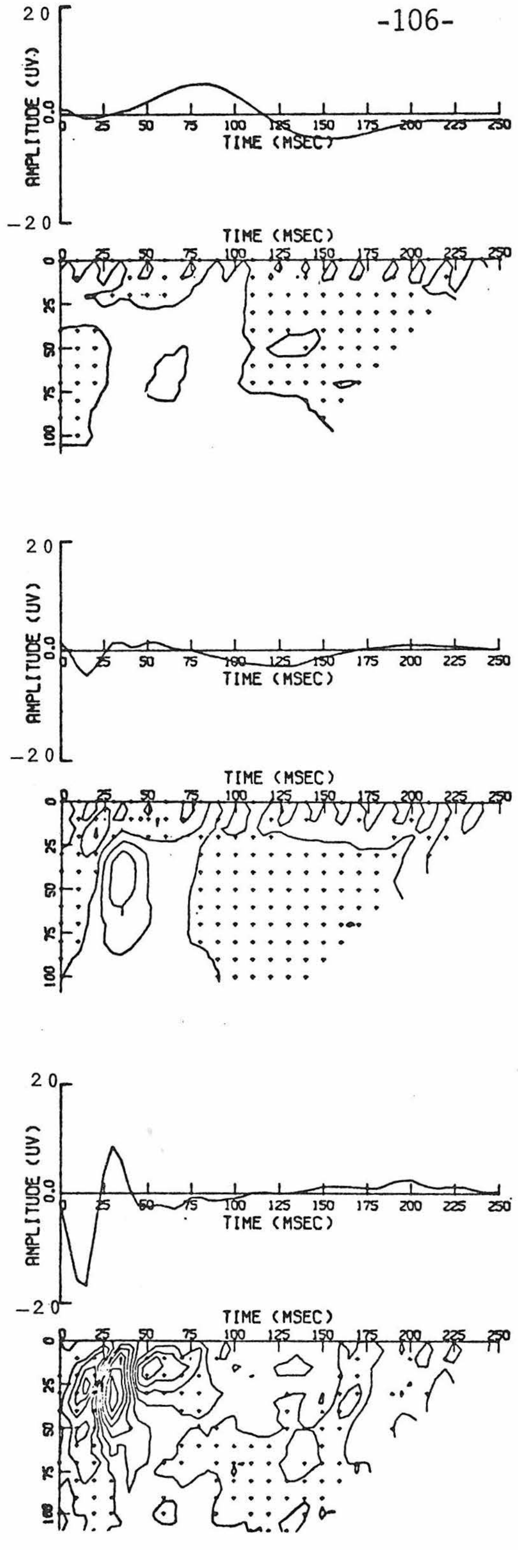


Figure 5C.1 Intensity series for normal monkey (KF).



≃-2 log units
(mostly scotopic)

≃-1 log units

≃0 log units
(mostly photopic)

Figure 5C.2 Intensity series for normal monkey (KF and KS). (KS scale = 0.25 μv/contour).

The interpretation of the components in these normal KF's is aided by the KS with its different emphasis on the different components of the ERG. The second dimension of the KS gives more scope in the interpretation. It is helpful in examining the normal ERG, but since it (in this case at least) maintains a more normal shape than the KF during retinal degeneration, it is even more helpful in examining the abnormal ERG's.

A progressive review of the degeneration of the retina as reflected in the ERG is seen in figures 5C.3 and 5C.4. These responses were obtained using the same intensity flash as the 0 log unit response of figures 5C.1 and 5C.2, but note that the scales are different.

The use of the KS is demonstrated by the ease with which the b-wave is identified in these highly abnormal ERG's. The amplitude of the b-wave is so markedly reduced for the later ERG's that it would be difficult to identify in the KF alone.

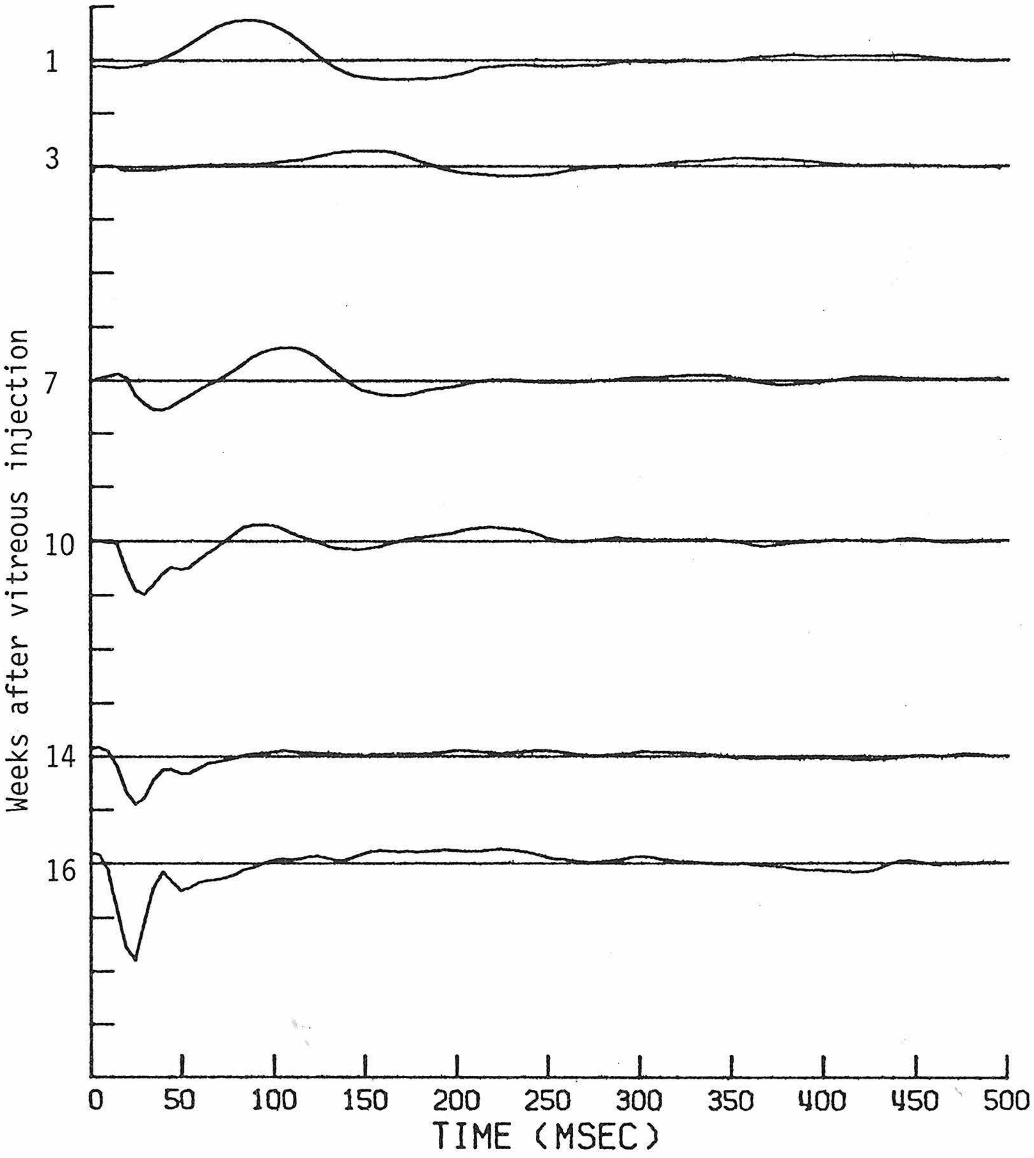


Figure 5C.3 Time series for vitreous injection (KF).

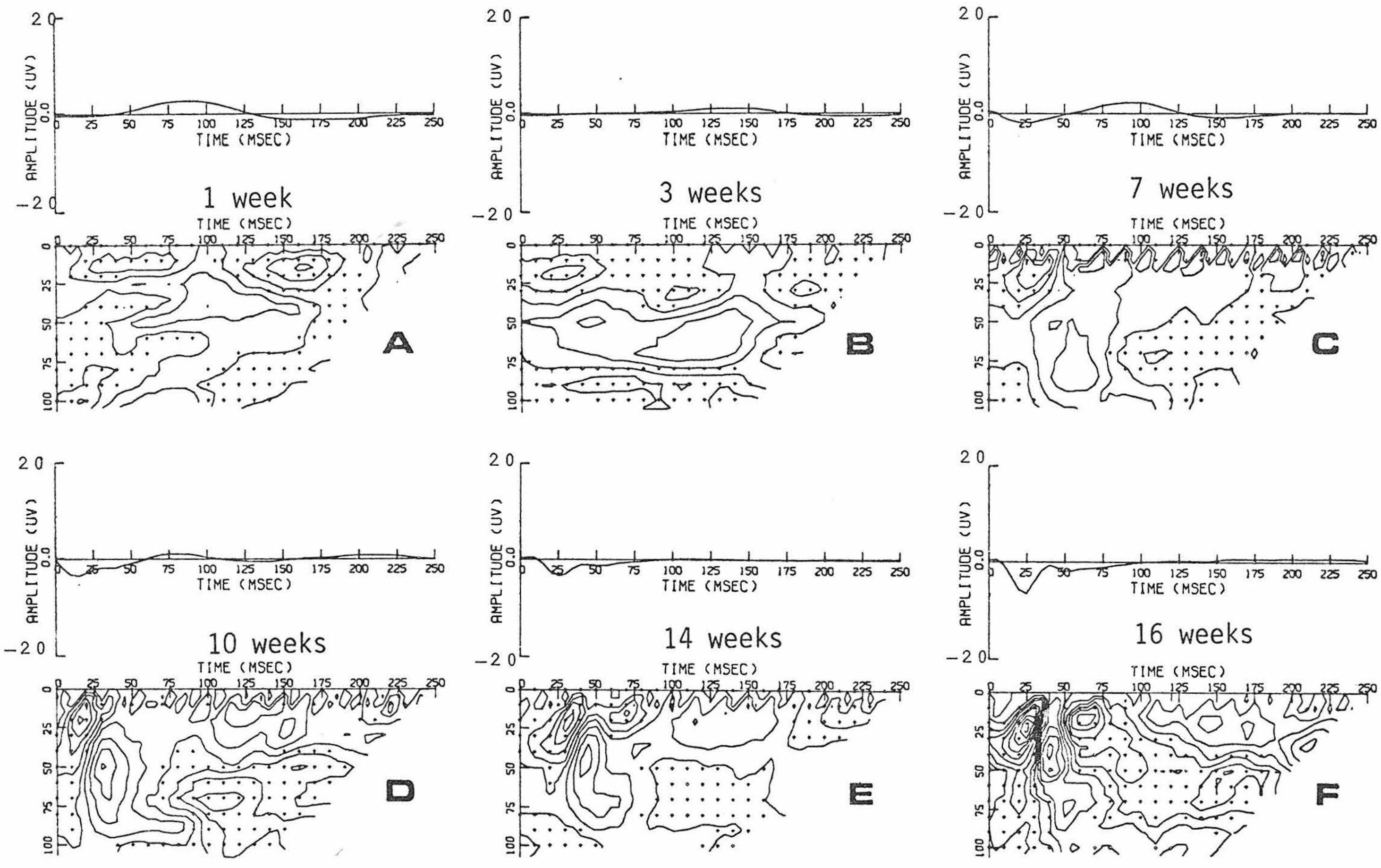


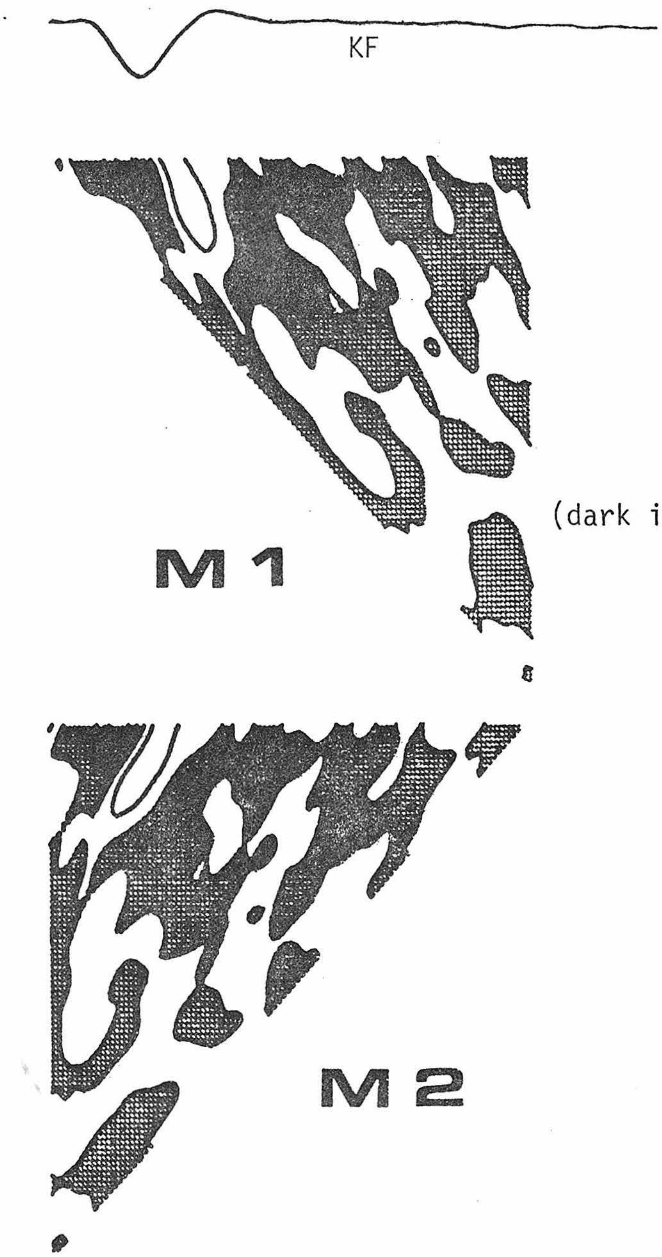
Figure 5C.4 Time series for vitreous injection (KS).

(KS scale = 0.125 μv/contour). Intensity = 0 log units.

5d -- saturation vs. adaptation

The KS has so far always been displayed in M2 (mode 2, section 3a), which is appropriate for the ERG. For some systems with different types of adaptation, a different mode of display is more appropriate. By displaying the KS in different modes one can be given an indication of the internal organization of the system under study. If the features of the KS are aligned vertically with analogous features in the KF when displayed in M2 (as the ERG does) this implies that the adaptation takes place before the part of the system which lends the characteristic shape of the KF to the response (figure 3A.8A). If, however, the features of the KS are aligned vertically with the KF when displayed in M1, this implies that the adaptation takes place after the basic processing which gives the KF its shape.

An example of a system which exhibits this 'shape followed by adaptation' organization, is the secondary cell of Drosophila melanogaster from which some kernels have been kindly donated by Robert Powers (Powers, 1979) and displayed in figure 5D.1. Notice that the main structure, in the upper left corner, is better aligned with the corresponding structure in the KF when displayed in M1 rather than M2. Also, look at the positive (non-speckled) part which extends downward from the top of the KS and note that the polarity of the KS is the same as that of the KF. This is different from the kernels for the ERG, and implies that the conditioning stimulus enhances the KF rather than suppressing it as in the ERG.



(dark is negative)

Figure 5D.1 M1 vs. M2 for saturation.

Kernels courtesy of Bob Powers, from Drosophila melanogaster secondary cell.

To help in the decision of whether to use M1 or M2, note the data displayed in figure 5D.2, which is an intensity series. The feature which is of interest is that the KF becomes faster (the peaks get closer to the origin) with increasing light. This basic effect should also be evident in the KS when displayed properly. That is, when the conditioning stimulus is closer to the test stimulus (thus having more effect) the response should be faster than when the two stimuli are widely separated. When displayed in M1 this is true; the enhancement seen in the KS moves toward the leading edge of the components in the KF as the stimuli approach each other (at the top of the KS). The opposite happens when the KS is displayed in M2, thus lending credence to the other mode.

The KS and the system are thus complementary in the sense that each expresses information about the structure of the other. This can be of use when first approaching a system and the most basic features need to be established.

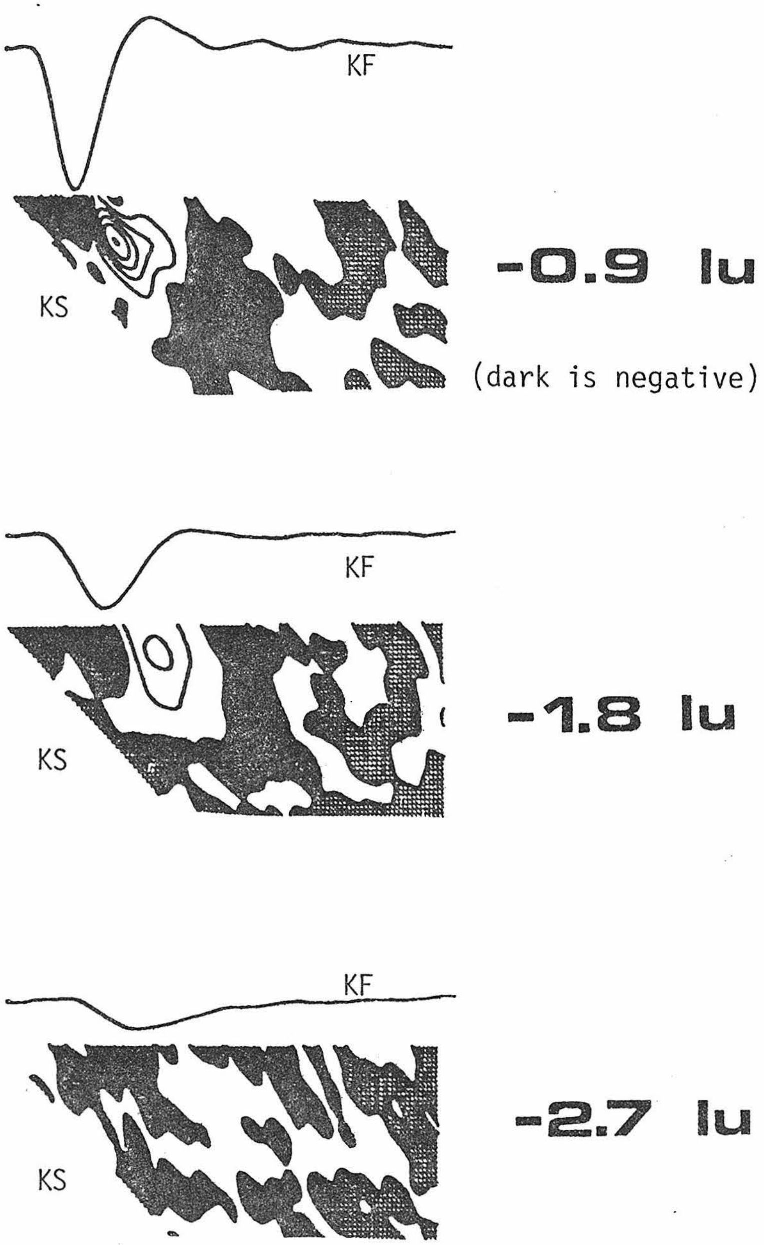


Figure 5D.2 Intensity series for saturation.

Kernels from *Drosophila melanogaster* (Bob Powers).

5e -- clinical uses

White noise and kernels explore the rapid adaptation of the retina which occurs too rapidly to be explored using conventional techniques. The data presented here explore the region down to 15ms. The region between 0 and 15ms may be explored with stimuli which have less power than a 15ms stimulus, but they require longer experiments. The stimulus used is a compromise between KS resolution and power.

The fact that this technique of augmenting the KF with the KS works for interpreting abnormal kernels is seen by comparing the kernels from normal subjects with those from patients with Retinitis Pigmentosa and Diabetic Retinopathy (R.P. and D.R.) and from monkeys with vitreal hemorrhages (section 5C). The KF's show the same effects as the conventional average flash responses and thus are of little additional value. However, when the KS are also taken into account, the additional information can be used to aid in the interpretation both of the KF and also in the determination of the cause of the abnormality.

We have collected a series of kernels from 15 normal subjects at six different intensities varying from mostly scotopic to mostly photopic. The mean and standard deviation of the mean for all kernels (15 KF and 15 KS) for each intensity were calculated and are displayed in figures 5E.1, 5E.2 and 5E.3. Since the shape of the kernel, and the KF to KS ratios are the most important features of the kernels, we removed the inter-subject amplitude variations (≃50%) by multiplying each subject's kernels by a single scale factor. This

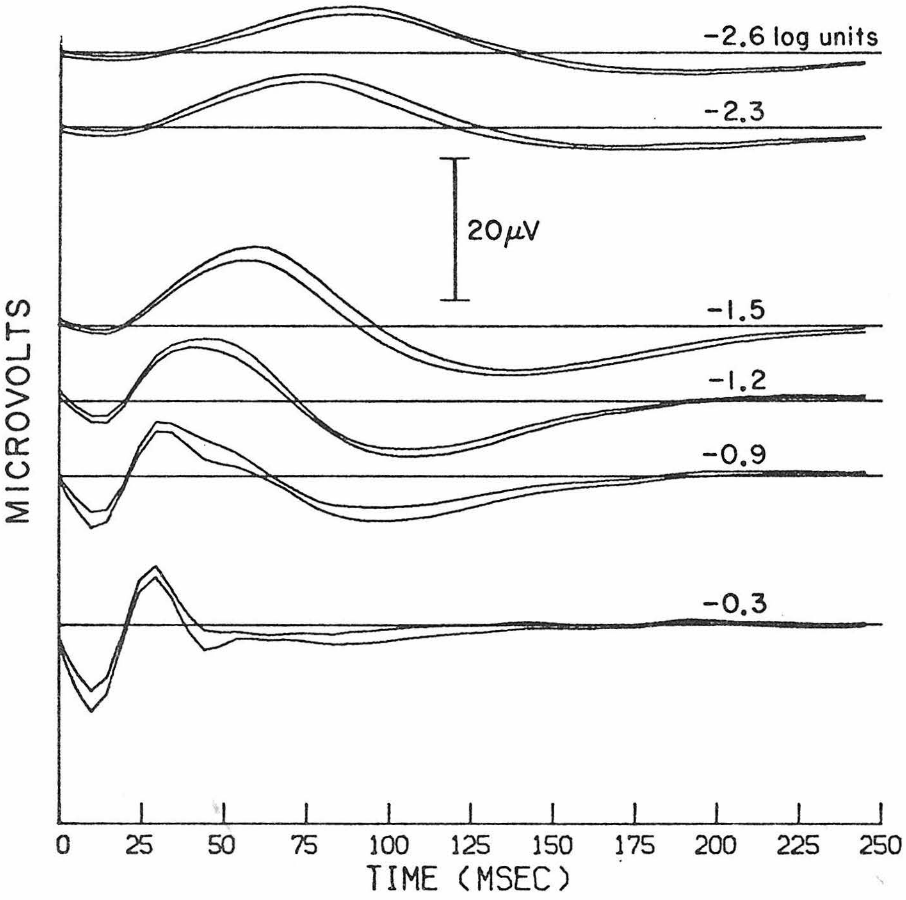
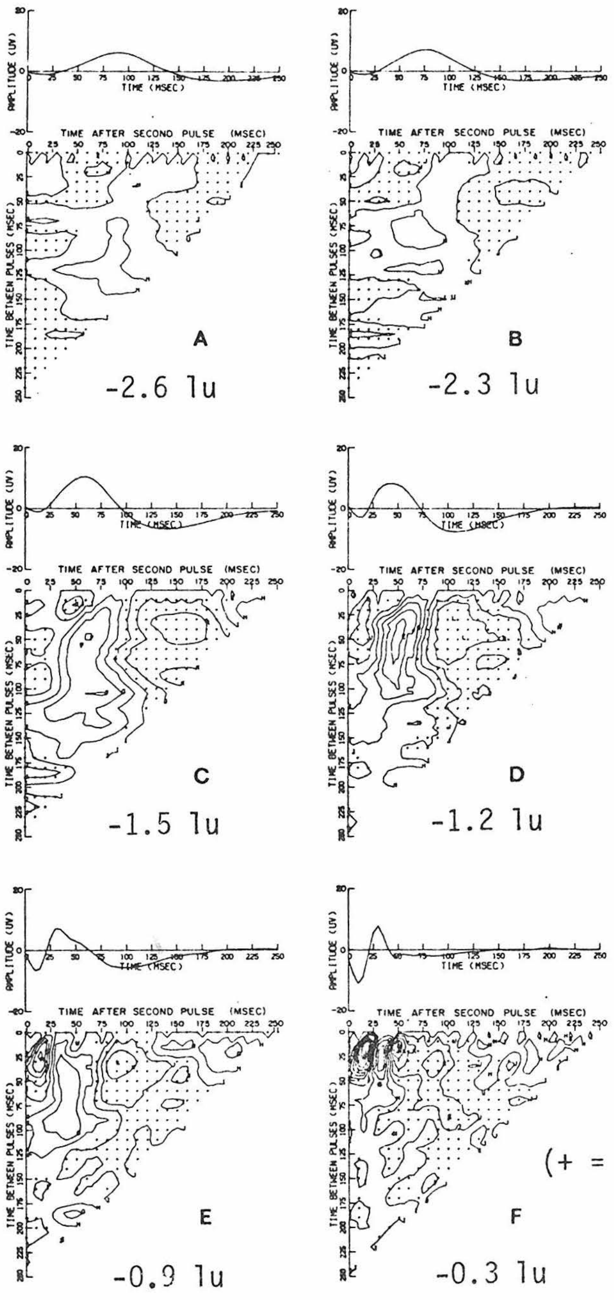


Figure 5E.1 KF intensity series with standard deviation.

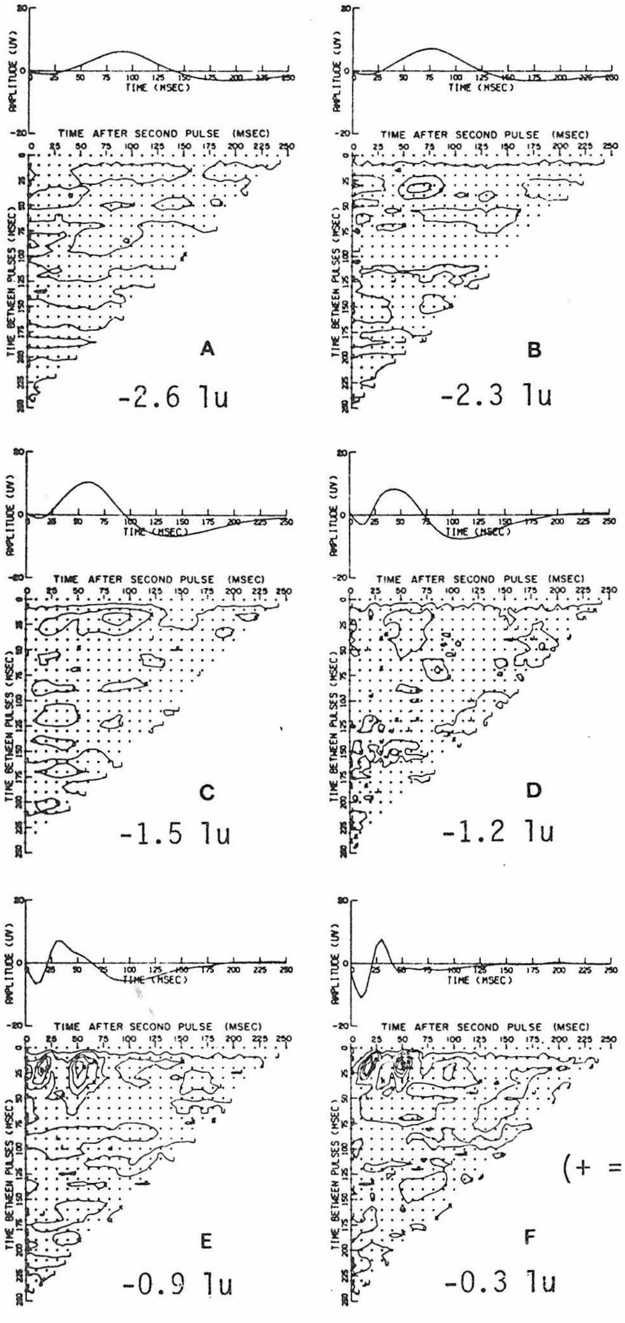
The mean response of 15 normal subjects, plotted as the mean plus one standard deviation of the mean and the mean minus one standard deviation of the mean. The intensities correspond to those shown in figure 5A.1.



(+ = positive)

Figure 5E.2 KS intensity series for normal subjects.

The mean KS corresponding to each of the mean KF's in figure 5E.1. (KS scale = 0.25 μv/contour).



(+ = positive)

Figure 5E.3 KS intensity series - standard deviations.

The standard deviation of the mean for the KS's of figure 5E.2. (KS scale = 0.0625 (1/16) μv/contour).

factor was chosen such that the -1.5 log unit KF matched a target kernel.

Except for the amplitude, the kernels for R.P. are normal. The KF and KS are smaller than those for normal subjects. These results can be interpreted for R.P. as meaning that the changes which are occuring in the retina are not affecting the function of those elements of the retina responsible for the generation of the ERG. This is compatible with the general feeling that the mechanism responsible for the reduction in amplitude is caused partially by a reduction in the area of functioning receptors and partially by a reduction of the resistivity of the pigment epithelium which would tend to shunt some of the current normally measured in the ERG. Both of these mechanisms would merely cause a decrease in the size of the ERG without causing any change in the shapes of the kernels.

In the case of the kernels from the patients with D.R. however, the kernels show more interesting effects. The KF exhibits a marked decrease in the amplitude of the b-wave without a corresponding decrease in the size of the a-wave (which is a reflection of the late receptor potential). In addition to this, the a-wave portion of the KS is fairly normal, but the b-wave portion is completely suppressed. The suppression of the b-wave in the KS is much more pronounced than the suppression of the b-wave in the KF.

The normal a-wave in both the KF and KS implies that the receptors are functioning normally. The suppressed b-wave in the KF implies that the b-wave generation mechanism is not, and the fact that the the b-wave is suppressed further in the KS than in the KF implies

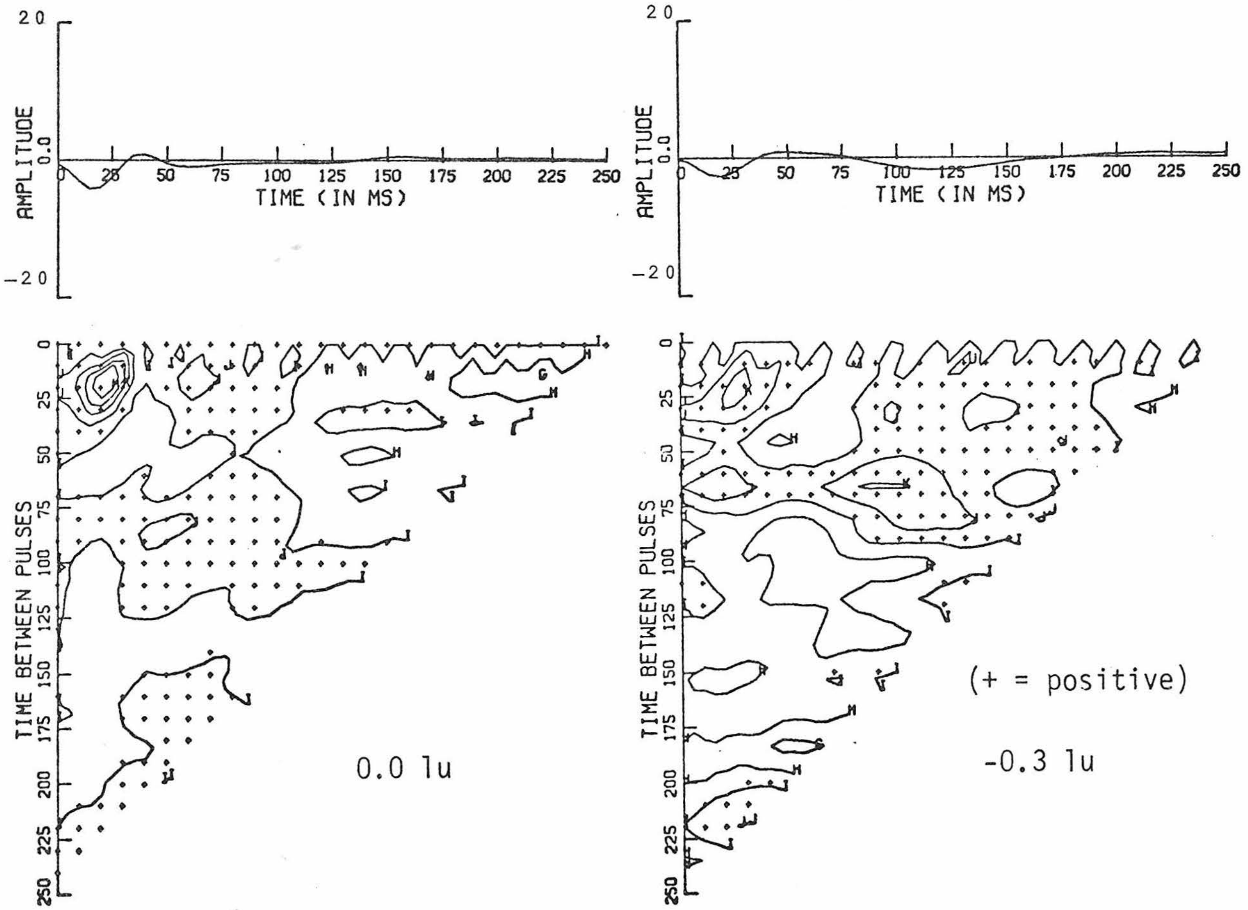


Figure 5E.5 KF and KS from a patient with Diabetic Retinopathy.

(KS scale = 0.2 µv/contour).

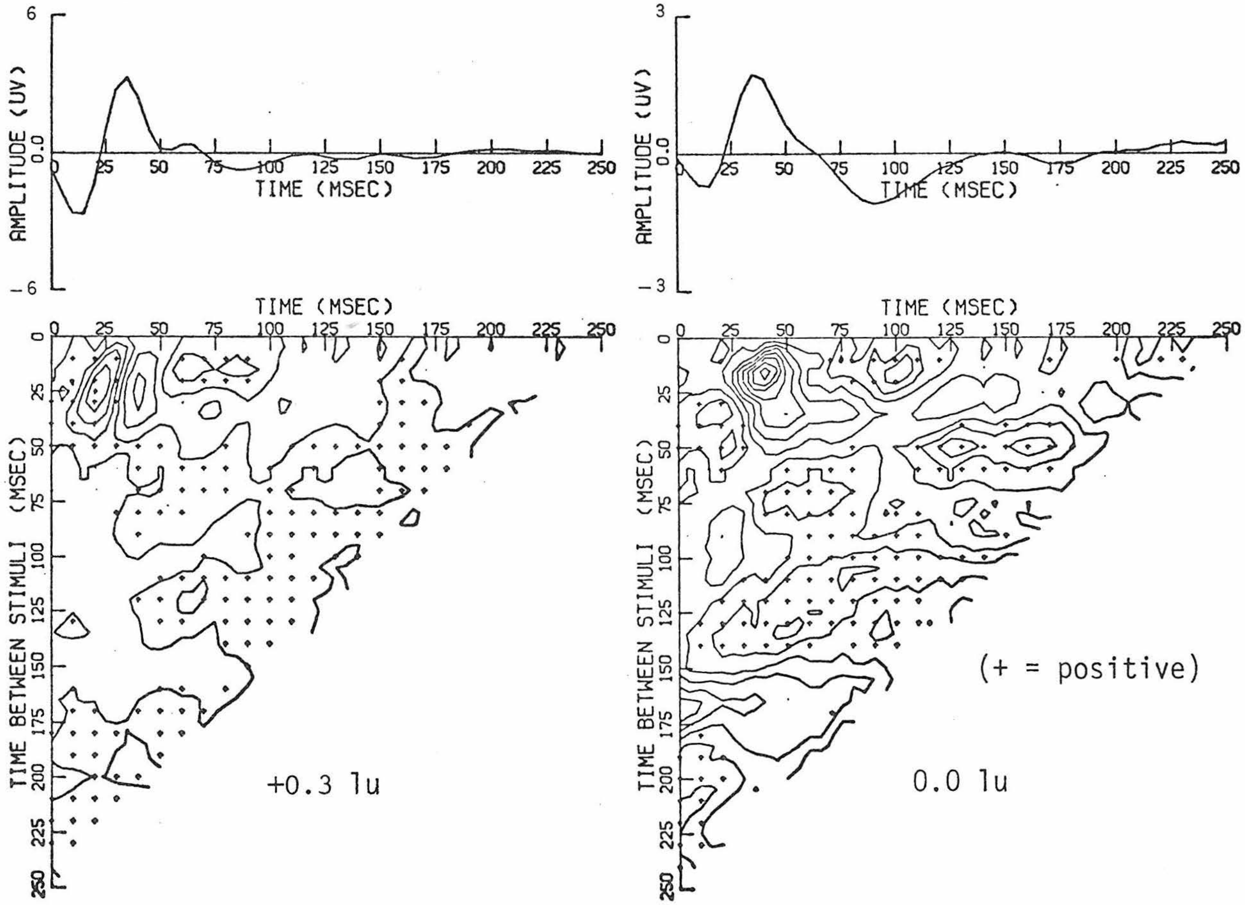


Figure 5E.4 KF and KS from a patient with Retinitis Pigmentosa.

Note that the scale factors are different, and that they are smaller than those for normal subjects. (KS scale = 0.2 µv/contour @+0.3 lu; = 0.05 µv/contour @0.0 lu).

that the suppression is not simply a reduction in the number of active b-wave generators, but that the abnormality in the retina is actually affecting the function of the b-wave generators. The conclusion is plausible when compared to the underlying pathology. The major effect of diabetes upon the retina is to cause rupture of some of the small retinal arteries with a resultant loss of blood supply (ischemia) to local areas of the retina (but not receptors since they are supplied from the choroid). The reduction in the amplitude of the KF b-wave can be explained in terms of this effect, but in addition to this, the extra reduction in the amplitude of the KS b-wave implies that the ischemia disrupts the adaptation process which occurs within the b-wave generators. This cannot be extracted from the KF or average flash response alone, but needs information from the KS.

Another example of an abnormal ERG is shown in figures 5C.3 and 5C.4 from a monkey with a vitreous injection of blood. The degeneration of the ERG with time is seen by comparing each of the abnormal kernels with the normal kernels.

The initial effect of the blood (from 1 to 3 weeks, approximately) is simply that it is nearly opaque and acts to reduce the intensity of the light reaching the retina. This produces fairly normal but scotopic responses. At around 7 weeks the blood (presumably) begins to clear allowing more light to reach the retina, thus causing the ERG to become larger with a hint of becoming more photopic. The ERG continues to become more photopic throughout the rest of the series; however the a-wave recovers much more fully than the b-wave.

After clearing, the primary effect of the blood is that the iron in the hemoglobin of the blood slowly poisons the retina from the inside out. The b-wave is poisoned first since the a-wave is generated by the receptors which are further from the vitreous.

The KS may be used the obtain a better idea of the type of changes occuring in the retina. The pattern of the KS (its outline, without regard to the amplitudes of the features) is consistent with the KF throughout, i.e. the KS changes from scotopic to photopic together with the KF. The amplitude of the b-wave in the KS is suppressed by the same amount (this is seen best in the later kernels). This implies (unlike the kernels from diabetic retinopathy) that the functioning of the b-wave generator is not being affected, but the there are probably fewer functioning b-wave generators.

In these three areas the KF and KS taken together give a more confident measure of the integrity of the retina than the KF or conventional average flash responses. They give clinical medicine an entry into functional properties of the retina so far inaccessible with the ERG.

In R.P. the amplitude of an otherwise normal ERG is smaller than normal, in the vitreous hemorrhage the retina retained normal function while fewer b-wave generators continued to function, and in D.R. only the receptors maintain normal function while the b-wave is suppressed and shows evidence of abnormal function as well.

5f -- conclusions

White noise kernels have been obtained and described which express certain properties of the photopic ERG and its adaptation. The series of first order kernels in figure 5A.1 exhibit the wide range of adaptation which the system can undergo. Each set of kernels (first, second and third order) express the dynamics of the adaptation for a given level of brightness. The following passage by Normann and Perlman (1979) gives a summary of the present understanding of adaptation in the visual system and will provide a framework for further discussion of adaptation.

> Light-adapting the retina by background illumination changes the sensitivity of the visual system. Measurements of the photoresponses of vertebrate photoreceptors have shown that much of the sensitivity change produced by background light has its origins in these cells. Two types of sensitivity changes have been described in both rods and cones; {one called response compression and the other called cellular adaptation.} Cellular adaptation is an active intrinsic mechanism which adjusts the dynamic range of the cell, allowing it to respond in a graded manner to light stimuli even in the presence of very bright ambient illuminations. Under any level of steady background illumination, both mechanisms may be operating. However, the relative contribution of each mechanism to the total loss of cell sensitivity is still in dispute amongst workers in this field.

Presumably cellular adaptation is responsible for maintaining the state of adaptation in the proper range, and response compression is the effect of the finite ability of the photoreceptor to respond to overloads. We wish to suggest in this discussion of our results that white noise kernels obtained at a given level of effective background illumination primarily represent the operation of cellular adaptation, without a large contribution from response compression. This is because adaptation as a whole adjusts the gross sensitivity of the system to place the background level of our stimulus in the operating range of the photoreceptor. The low power of the white noise stimulus does not contain excursions large enough to probe the response compression, but instead merely has enough power to probe the active cellular adaptation on a continuous basis.

It seems likely that this active cellular adaptation uses information about how well it is adjusting the sensitivity of the photoreceptor to match the incoming light stimulus with the allowable range of the system. We would like therefore to suggest that the cellular mechanism is feedback oriented.

The b-wave model as it has been presented does not use feedback sensitivity control, so we propose to change the arrangement of the model as shown in figure 5F.1. The interesting aspect of the b-wave model is the adaptation, since little is said about the b-wave generation (modeled by a linear element defined by the shape of a typical b-wave). Since much of the adaptation takes place in the photoreceptors, we will apply the b-wave model to a model of the LRP. This is done by replacing the b-wave generator with an LRP generator (similarly defined by the shape of a typical LRP).

When the model is rearranged to provide feedback sensitivity control, the model then accounts for the polarity of the KT and

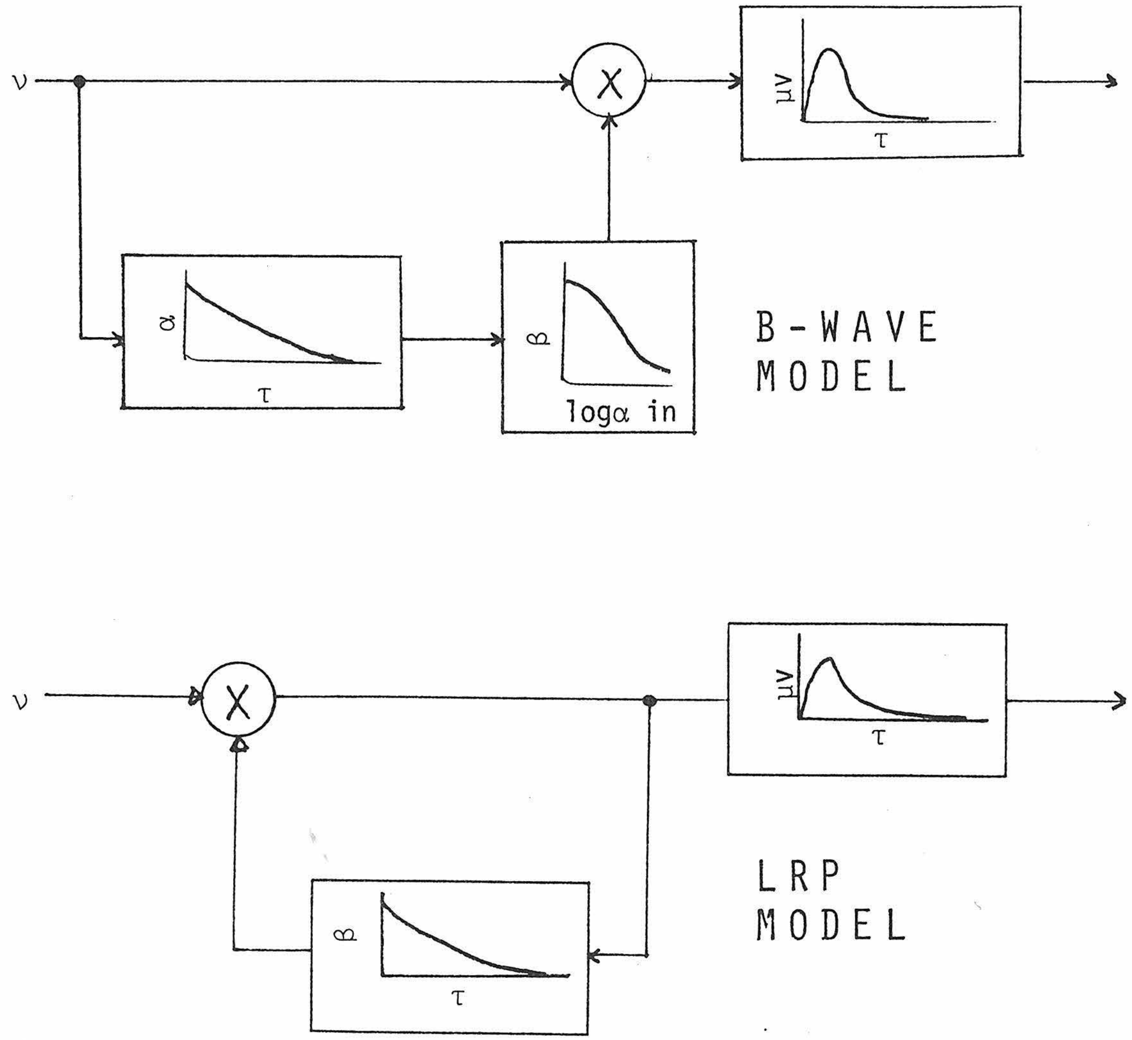


Figure 5F.1 B-wave model vs. LRP model.

therefore accounts for suppression-recovery. The amount of suppression which is left behind each flash to affect the following flashes is now proportional to the size of the attenuated flash rather than the unattenuated input flash (as it was in the b-wave model). Thus the second flash leaves behind much less suppression than the first. The third flash is then not reduced as much as the second.

In the new arrangement the feedback from the sensitivity adjusting mechanism represents the ability of the system to monitor the present match between the input light and the output of the cellular adaptation mechanism. The non-time-varying function of the b-wave model is not included in the LRP model because it is used to account for response compression which is not probed by our low power white noise stimulus.

It has often been stated that the ERG is very nearly linear, which is true when low power stimuli such as white noise are used. However, because the nonlinear effects are small does not imply that they are not useful, on the contrary, most of the interesting effects are in the nonlinearities, in this case the details of the photopic rapid adaptation.

Appendices
(Some details.)

Appendix I (auto- and cross-correlations)

For a complete account of the history and mathematical basis which underlies the white noise technique, see the book Analysis of physiological systems by Panos and Vasilis Marmarelis. A very condensed set of formulae is gathered here for reference.

Cross-correlation is used to extract the impulse response from a white noise stimulus and the response to that stimulus. It is a general form of signal averaging.

$$C(\tau) = 1/N * \sum_{i=1}^{N} S(i) * R(i + \tau)$$

where:

S(i)	is the i-th sample from the stimulus
R(i)	is the i-th sample from the response
N	is the total number of samples in the record
C(τ)	is the value of the cross-correlation of the stimulus with the response τ samples from the origin (Equivalent to the response τ samples after an impulse.)

Auto-correlation is exactly the same as cross-correlation, but with the stimulus placed in the formula for both the stimulus and the response.

To convert the cross-correlation to the traditional first order kernel one must scale it by the power of the stimulus. (Throughout, the terms power and power density will be used synonomously.)

$$KF(\tau) = 1/L * C(\tau)$$

where:

$KF(\tau)$	is the value of the first order kernel
L	is the power of the stimulus, defined below

$$L = \delta * \sum_{\tau=-\omega}^{\omega} C(\tau)$$

where:

δ	is the time between samples
N	is the total number of samples in the record
$C(\tau)$	is the auto-correlation of the stimulus
ω	is the half-width of the autocorrelation (The autocorrelation of the stimulus should be zero outside of the half-width, but to keep random fluctuations from affecting the estimate of the power the summation should end at the half-width.)

Note: The power of the stimulus is merely the integral of the auto-correlation function. It may also be approximated by the height of the auto-correlation multiplied by the half-width.)

$$L \text{ (alternate)} = C(0) * \omega$$

Second order kernels are computed in a similar manner, The correlation is between the response and two samples of the stimulus at various inter-stimulus intervals.

$$KS(\tau_1, \tau_2) = \tfrac{1}{2} * 1/L^2 * \sum_{i=0}^{N} S(i - \tau_1) * S(i - \tau_2) * R(i)$$

where:

$\frac{1}{2}$	is due to the symmetry of the KS
$1/L^2$	is used if the kernel is to be scaled by the power of the stimulus
$KS(\tau_1, \tau_2)$	is the second order kernel (in mode 0, M0)

To see how cross-correlation is related to signal averaging, look at the cross-correlation function differently. Rather than summing over time for each τ separately, consider the following where the whole cross-correlation function is updated at each time period. The normalization by N does not take place until the end of the process. (Although it can be done in a manner similar to calculating a running average of numbers.)

$$C(0 \dashrightarrow \tau)_{new} = C(0 \dashrightarrow \tau)_{old} + (S(i) * R(i \dashrightarrow \tau))$$

Now suppose that S(i) only had values of 0 or 1 corresponding to no-flash or flash, respectively. When the stimulus was 0, nothing would be added to the C_{new}, just as in signal averaging. When a flash occurs, the stimulus becomes 1 for one sample and the response which follows the flash is added into the correlation function, just as the response is added into the average in a signal averager. The difference is that correlation can average signals which overlap, whereas signal averagers cannot.

Appendix II (convolution)

Convolution is used to predict the response to a given stimulus when the first order kernel is already known, and is thus complementary to correlation which is used to extract the first order kernel from a stimulus and a response.

$$\begin{aligned} R(i) \;=\; & \delta * \sum_{\tau=0}^{T} KF(\tau) \;*\; S(i-\tau) \\ & + \delta*\delta \;*\; \sum_{\tau_1=0}^{T} \sum_{\tau_2=0}^{T} KS(\tau_1, \tau_2) \;*\; S(i-\tau_1)*S(i-\tau_2) \\ & - L \;*\; \delta \;*\; \sum_{\tau=0}^{T} KS(\tau, \tau) \end{aligned}$$

where:

$R(i)$	is the i-th response sample which is predicted
$S(i)$	is the i-th stimulus value
$KS(\tau_1, \tau_2)$	is the second order kernel
T	is the length of the kernels in sample periods
L	is the power of the stimulus (as in correlations) (Note that for binary stimuli the KS is undefined on the main diagonal ($\tau_1 = \tau_2$) and this aspect of the formula is unused ($KS(\tau, \tau)$).)

REFERENCES

Arden G., R. Granit and F. Ponte. 1960. Phase of suppression following each retinal b-wave in flicker. J. Neurophys. 3:305 - 314

Armington, J. 1974. The electroretinogram. Academic Press, New York.

Barker, H. and T. Pradisthayon. 1970. High-order autocorrelation functions of pseudorandom signals based on m-sequences. Proc. IEE. 117:1857 - 1863

Barker, H., S. N. Obidegwu and T. Pradisthayon. 1972. Performance of antisymmetric pseudorandom signals in the measurement of 2nd-order Volterra kernels by crosscorrelation. Proc. IEE. 119:353 - 362

Barker, H. and S. N. Obidegwu. 1973. Combined crosscorrelation method for the measurement of 2nd-order Volterra kernels. Proc. IEE. 120:114 - 118

Brown, K.T. 1968. The electroretinogram: its components and their origins. Vision Res. 8: 633 - 677

Burian, H. M. and L. A. Allen. 1954. A speculum contact lens for electroretinography. Electroencephalography and Clinical Neurophysiology. 6:509 - 511

Dowling, J. E. 1967. The site of visual adaptation. Science. 155:273 - 279

Fricker, S. J. and J. J. Sanders. 1975. A new method of cone electroretinography: the rapid random flash response. Invest. Ophthal. 14:131 - 137

Karwoski, C. J. and L. M. Proenza. 1977. Relationship between Müller cell responses, a local transretinal potential, and potassium flux. J. Neurophys. 40:244 - 259

Kline, R. P., H. Ripps and J. E. Dowling. 1978. Generation of b-wave currents in the skate retina. Proc. Natl. Acac. Sci. USA. 75:5727 - 5731

Koblasz, A. J. 1978. Nonlinearities of the human ERG reflected by Wiener kernels. Bio. Cyber. 31:187 - 191

Marmarelis, P. Z. and V. Z. Marmarelis. 1978. Analysis of physiological systems. Plenum Press. New York.

Marmarelis, V. Z. 1978. Random versus pseudorandom test signals in nonlinear-system identification. *Proc. IEE.* 125:425 - 428

Newman, E. A. 1979. B-wave currents in the frog retina. *Vis. Research.* 19:227 - 234

Normann, R. A. and I. Perlman. 1979. Evaluating sensitivity changing mechanisms in light-adapted photoreceptors. *Vis. Research.* 19:391 - 394

Owen, W. and A. J. Sillman. 1973. The suppression-recovery effect in the frog photoreceptor. *Vis. Research.* 13:2591 - 2594

Powers, R. L. 1979. Iontophoretic studies of visual systems in *Drosophila melanogaster.* Ph. D. Thesis, California Institute of Technology.

Ream, N. 1970. Nonlinear identification using inverse-repeat m sequences. *Proc. IEE.* 117:213 - 218

Rodieck, R. W. 1972. Components of the electroretinogram -- a reappraisal. *Vis. Research.* 12:773 - 780

Rodieck, R. W. 1973. The vertebrate retina. *Principles of structure and function.* W. H. Freeman and Company, San Fransisco

Salinger, W. L. and D. B. Lindsley. 1971. The suppression-recovery effect and visual adaptation in the cat. *Vis. Research.* 11:1435 - 1444

Salinger, W. L. and D. B. Lindsley. 1972. The suppression-recovery effect in relation to stimulus repetition and rapid adaptation. *Vis. Research.* 12:1897 - 1905

Troelstra, A. 1971. Harmonic distortion in the frog's ERG and its possible relation to differences in latencies. *Vis. Research.* 11:393 - 403

Troelstra, A. and N. M. J. Schweitzer. 1968. Nonlinear analysis of electroretinographic b-wave in man. *J. Neurophysiol.* 31:588 - 606

Werblin, F. S. and J. E. Dowling. 1969. Organization of the retina of the Mudpuppy, *Necturus maculosus.* II. Intracellular recording. *J. Neurophysiol.* 32:339 - 355

Williams, R. A. 1977. The processing of velocity information by the pursuit occulomotor system. Ph. D. Thesis, California Institute of California