1. TITLE OF PROJECT
Effects of V1 feedback on LGN function

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR
Kaplan, Ehud

3a. NAME (Last, first, middle) Kaplan, Ehud
3c. POSITION TITLE Professor
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT Ophthalmology
3f. MAJOR SUBDIVISION School of Medicine
3g. TELEPHONE AND FAX (Area code, number and extension) TEL: 212-241-9607 FAX: 212-289-5945

4. HUMAN SUBJECTS
4a. Research Exempt No Yes
4b. If "Yes," Exemption number M-1155
4c. NIH-defined Phase III Clinical Trial
5a. If "Yes," IACUC approval Date pending
5b. Animal welfare assurance number

6. DATES OF PROPOSED PERIOD OF SUPPORT (month, day, year-MM/DD/YY)
From 04/01/05 Through 03/31/10

7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD
7a. Direct Costs ($) 250,000.
7b. Total Costs ($) 423,750.

8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT
8a. Direct Costs ($) 1,250,000.
8b. Total Costs ($) 2,118,750.

9. APPLICANT ORGANIZATION
Name Mount Sinai School of Medicine
Address One Gustave L. Levy Place, Box 1075 New York, NY 10029-6574

10. TYPE OF ORGANIZATION
Public: Federal State Local
Private: X Private Nonprofit
Forprofit: General Small Business
Woman-owned: Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
DUNS NO. (if available) 78861598
Congressional District 14

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name Ms. Jessica Moise
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Mount Sinai School of Medicine
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New York, NY 10029-6574
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E-Mail grants@mssm.edu

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Tel (212) 659-8970 FAX (212) 876-6789
E-Mail grants@mssm.edu

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE
I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

15. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE
I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF PI/PD NAMED IN 3a.
(In ink. "Per" signature not acceptable.)
DATE 05/26/04

SIGNATURE OF OFFICIAL NAMED IN 13.
(In ink. "Per" signature not acceptable.)
DATE 05/26/04

PHS 398 (Rev. 5/01)
The long range goal of our research is to understand how thalamic neurons integrate their various feedforward and feedback inputs, and what role these inputs play in the flow of information from retina to cortex through the thalamus. Most of the inputs and synapses in the mammalian lateral geniculate nucleus (LGN) are extraretinal, but the way in which these diverse inputs are integrated to control the flow of visual information from retina to cortex is not understood. In particular, the influence of the descending inputs from the cortex and the perigeniculate nucleus (PGN) on the spatio-temporal properties of receptive fields of LGN relay neurons is unknown.

To address this gap in our knowledge, we shall study the temporal and spatial aspects of receptive fields in monkey LGN before and during inactivation of the cortical feedback to the LGN. The dynamical properties will be probed with a double m-sequence stimulation paradigm, which will provide new information about both the linear and non-linear dynamics of these neurons, and will expose the effects that the feedback from V1 has on this dynamical behavior. Our hypothesis is that the corticofugal feedback to the LGN has a significant effect on four specific aspects of LGN function: dynamics, receptive field organization, transmission from retina to cortex and response gain.

The proposed studies will furnish new information about the effects of the corticofugal pathway on several important dynamical and spatial parameters of the receptive fields of LGN relay cells. These findings will extend and deepen our understanding of the function of this massive yet elusive neural pathway, and pave the way for realistic modeling of the early stages of the visual system. Because such descending pathways are ubiquitous in the brain, the findings are likely be relevant to other reciprocally connected brain regions.
Principal Investigator/Program Director (Last, first, middle):

RESEARCH GRANT

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Appendices NOT PERMITTED for Phase I SBIR/STTR unless specifically solicited.

Number of publications and manuscripts accepted for publication (not to exceed 10) .... 6

Other items (list):
7. Letter of agreement from

☐ PHS 398 (Rev. 05/01)
BUDGET JUSTIFICATION PAGE
MODULAR RESEARCH GRANT APPLICATION

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Personnel

Ehud Kaplan, Ph.D., Principal Investigator (Effort):
Kaplan will direct the overall project, and will take part in all its aspects: experiments, data analysis, manuscript preparation, and so on. He has been working on the mammalian visual system since 1973, and for the past several years has been using optical imaging and electrophysiological recordings in studies of the visual cortex. He has appointments in the departments of Ophthalmology, Physiology & Biophysics. We are requesting --- of his salary on the current grant

Ph.D., Investigator (Effort):
Dr. is an accomplished neurophysiologist, with much experience in working on the primate cortex, in both electrophysiological and optical measurements. Each experiment requires many man-hours, not only during the experiment itself, but also for subsequent analysis of the many gigabytes of data, reprogramming of analysis programs, new stimuli and data acquisition protocols, so we need to work in teams. We are asking for --- of the salary of Dr.

Systems Administrator (Effort):
(Computer specialist): is crucial for the maintenance of the hardware and software which we use, especially the stimulation and data acquisition systems. He is an Electronics Specialist at The Mount Sinai School of Medicine. We are requesting --- of his salary on this grant.

To be named, Post Doctorial Fellow (Effort):
We shall recruit a post-doctoral fellow with training in computational neuroscience, to carry out the simulations for Specific Aim A2, guided primarily by We have had much experience in finding post-doctoral fellows and training them for this kind of modeling work.

Consortium

Fee (SBIR/STTR Only)
8 pages redacted--biosketches omitted as requested
RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under “Other,” identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:
Dr. Kaplan’s lab includes approximately [blank space] at Mount Sinai School of Medicine. Additional space is available for storage, electrode preparation, and miscellaneous use.

Clinical:
NA

Animal:
Our animals are housed in the [blank space]. They receive expert veterinarian care from a professional staff, supervised by Dr. [blank space].

Computer:
We have numerous PCs in the lab for data acquisition, stimulus generation and image analysis, including one with 720 gb and another with 320 gb of disk storage for image acquisition. The computer simulations will be performed on an SGI ORIGIN computer, which has 8 processors and 12 gB of RAM. We have constructed a 16 node BeoWulf cluster for the more demanding computations that will be involved in modeling larger cortical regions. It is already functioning in our lab.

Office:
Offices for the PI [blank space], the other investigators [blank space], the Post-Doctoral fellows, and the computer programmer are all available in the [blank space].

Other:
Well-equipped machine and electronics shops, together with a full-time machinist and an electronics engineer are supported by a core grant from the Eye Institute. They provide services to several laboratories in the Ophthalmology and Physiology departments. Histology service is also provided by the Core grant.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

We have a PixelVision Pluto CCD camera, a Pritchard light meter, several large disks and tape drives for data storage, a hood for perfusing the animals after the experiment, and the usual life support system for maintaining a mammal in a neurophysiological experiment, together with the electrophysiological equipment needed to collect neuronal responses: amplifiers, oscilloscopes, audio-monitors, micromanipulators, etc.
A  SPECIFIC AIMS

The LGN receives its primary excitatory input from retinal ganglion cells. However, a massive feedback pathway from V1 provides a significant fraction of the synapses to LGN relay cells. The role of this feedback in controlling LGN behavior is poorly and incompletely understood. In studying the effects of this feedback, we should bear in mind that there are significant nonlinearities in cortical processing, and therefore a comprehensive examination of the effects of cortical feedback on the LGN requires a study of both linear and nonlinear aspects of LGN function.

To address this gap in our understanding of the role of the cortical feedback to the LGN, we shall test the hypothesis that the corticofugal feedback to the LGN has a significant effect on four specific aspects of LGN function: dynamics, receptive field organization, transmission to cortex and response gain (contrast dependence). We shall record from LGN relay neurons in macaque monkeys, and use the double m-sequence stimulation paradigm (Benardete & Kaplan, 1997a,b) to accomplish the following specific aims:

1. Dynamics: Using reversible inactivation of the V1 feedback to LGN, study its effect on the linear and non-linear dynamics of LGN receptive fields using three approaches:

   (a) Modulate a small center spot and a larger surround annulus with a double m sequence stimulus.

   (b) Use cone isolating stimuli, modulated by a double m sequence, in 'type I' parvocellular cells, to excite the center and surround separately.

   (c) To maximize the activation of layer 6 neurons (which provide the feedback pathway), use oriented stationary gratings whose contrast is modulated in time by the double m-sequence stimulus.

2. Space: Using reversible inactivation of the V1 feedback to LGN, study its effect on the receptive field organization (including its 'extra-classical' components), using an m sequence modulated checkerboard.

3. Transmission: Investigate the impact of V1 feedback on the transmission of visual signals from retina to LGN by recording LGN output spikes together with their retinal inputs (in the form of slow synaptic ‘S’ potentials (Kaplan & Shapley (1984))).

4. Gain: Investigate the impact of cortical feedback on LGN gain by repeating the measurements at several contrasts (whenever recording stability permits).

B  BACKGROUND and SIGNIFICANCE

We briefly review the basic anatomy and physiology of the relevant circuits, point out serious gaps in our knowledge, and comment on the planned experimental approaches that are designed to fill those gaps.
B.1 Anatomical circuitry: the LGN and its network

The LGN contains both relay cells and intrinsic inhibitory interneurons, and at its surface a thin layer of inhibitory perigeniculate neurons, which are part of the thalamic reticular nucleus (TRN). Relay cells are of the ON and OFF types, with circular receptive fields, and center-surround antagonism. Each LGN cell receives forward excitatory input from a small number of retinal ganglion cells of the same functional type (ON or OFF), and inhibitory input from interneurons that themselves are excited through retinal ganglion cells and layer 6 afferents (see Sherman & Guillery (2003) for review). Inhibitory input is also provided from perigeniculate neurons that are excited through relay cell afferents on their way to the cortex and through layer 6 afferents on their way back to the LGN (Gilbert & Kelly (1975), Uhlrich et al. (1991)). These afferents form a part of the feedback from V1 that is carried by layer 6 axons, and which also provides excitatory input to relay cells. The feedback is anatomically massive, with 40 times more corticogeniculate than retinogeniculate contacts (Sherman & Koch (1986)).

In the 6 layers of the macaque LGN, relay cells are usually classified into 3 major populations: parvocellular (top 4 layers), magnocellular (bottom 2 layers) or koniocellular (scattered between the layers) (see Kaplan (2003) for a recent review). The connections between the LGN and the cortical regions are arranged topographically.

In addition to the forward input from the retina and the feedback from V1, the relay cells, interneurons, and perigeniculate neurons all receive modulatory input from the brainstem (Smith et al. (1998), Bickford et al. (1993), Ozaki et al. (1996), Ozaki et al. (1997a)). The circuitry is shown schematically in Figure 1.

---

**Figure 1: A schematic connectivity diagram for the LGN.** Different neurotransmitters are indicated by color. *Modulators* are shown by dotted lines, while *drivers* are shown in solid lines. (From Sherman & Guillery, 1998, 2003).
B.2 Space-time receptive field maps: m-sequences and reverse correlations

It is possible to study some spatial and temporal parameters of a system using sinusoidal stimulation at various spatial and temporal frequencies. However, the pseudo-random stimulation approach, which uses the reverse correlation method (introduced for receptive field mapping by Jones and Palmer (1987)), provides a more complete characterization of the receptive field than the more common measurement of either temporal or spatial properties alone (see also Reid et al. (1997) and Cai et al., (1997)). In addition, it provides information that is not available when the temporal or spatial characterizations are carried out separately. This method allows one to determine whether the receptive field is space-time separable, namely, whether the response can be expressed as \( R(x, y, t) = f(x, y) \cdot g(t) \) (Dawis et al. (1984), Jones & Palmer (1987), DeAngelis et al. (1993)). To determine in an efficient way the influence of the V1 feedback on the dynamical mechanisms that shape LGN function, we shall use in our studies the reverse correlation technique with a special variant of the m sequence method, the double m-sequence stimulation paradigm (see Benardete & Kaplan (1997b), Benardete & Victor (1994)). The results will shed new light on the details of the LGN receptive field structure, and will also provide the necessary information for the subsequent construction of more biologically realistic LGN models.

B.3 Biophysical machinery: the LGN ionic repertoire

Neurons in the mammalian thalamus possess, in addition to the usual complement of ionic channels found elsewhere in the brain, the somewhat unusual T-type \( Ca^{++} \) channel (Llinàs & Jahnsen (1982), Jahnsen & Llínàs (1984a,b), Huguenard & McCormick (1992)). This channel is inactivated when the neuron is depolarized, and is de-inactivated during hyperpolarization, leading to a bursty LGN discharge, unlike the tonic activity common during depolarization. Therefore, any input that influences the average membrane potential can have a profound effect on the dynamics of LGN relay cells. Since the bursts are due to regenerative calcium spikes, they represent a clear form of nonlinearity in LGN dynamics.

B.4 LGN Dynamics

To a first (crude) approximation, LGN dynamics are similar to those of its main excitatory input, the retinal ganglion cells (but see Kaplan et al. (1993), Mukherjee & Kaplan (1996, 1998), Funke & Eysel (1992), Wörgötter et al. (1998)). However, the extensive and numerous non-retinal inputs, together with the thalamic biophysical machinery and the intrinsic geniculate circuitry, all combine to generate important differences between the dynamical properties of the LGN input and output. The non retinal inputs to the LGN are mostly modulators, rather than drivers, in the terminology of Sherman and Guillery (1998), and they engage both ionotropic and metabotropic receptors (see, for example, Hohnke et al. (2000)), with fast and slow time constants, respectively. This fact alone suggests that the corticofugal pathway will have a significant effect on the dynamical properties of LGN relay cells, in addition to the effect exerted through the control of membrane potential, which determines the state of activation of the T channel mentioned above.

Nonlinearities: Benardete & Kaplan (1997b) have documented some dynamical nonlinearities in primate LGN neurons, and others have reported nonlinearities in cat LGN cells (for example, Funke et al. (1996)). Nonlinearities involving a suppressive surround have been reported by Wiesel & Hubel...
Principal Investigator/Program Director (Last, First, Middle) Kaplan, Ehud

(1966) in the primate LGN, by Kaplan & Shapley (1989) in P retinal ganglion cells and by Dubin & Cleland (1977) in the cat LGN. Our study will determine whether the cortical feedback plays a role in creating or augmenting the LGN suppressive surround.

Transmission: Even from the short record presented in figure 2 it is obvious that not all the retinal spikes are relayed to the visual cortex by the gate keepers at the LGN. Which spikes are deleted, and under what circumstances, depends on various factors, which include stimulus parameters (Kaplan et al. (1987)) and the state of the animal (Kaplan et al. (1993)). Because initial transients are more likely to be relayed faithfully, the LGN output is often more transient than its retinal input. For recent comprehensive reviews, see Victor (1999) and Kaplan & Benardete (2001).

Spike Timing and precision: A related but distinct topic is the issue of spike timing and temporal codes. An examination of panel C in Figure 2 shows the scatter of spike timing of the LGN relay neuron relative to its retinal drive. Virtually nothing is known about the effects of the corticothalamic feedback pathway on the precision of spike timing or on the information content of spike trains ascending from the LGN to V1, although recent evidence indicates that these issues are important for information processing in sensory system, since the precise timing of spikes affects their efficacy (see, for example, Usrey (2002)).

B.5 Non-retinal influences on thalamic function

B.5.1 - V1 feedback to LGN

How does the corticofugal pathway shape LGN processing, and to what purpose? This topic has been investigated repeatedly, mostly in cats and monkeys, but the research has produced mixed and sometimes conflicting results (see Singer (1977) and Alitto & Usrey (2003) for reviews). Nevertheless, a general modulatory role is clear, as in cat binocular vision (Schmielau & Singer (1977)), in temporal response (McClurkin et al. (1994), Marrocco et al. (1996)), in spatial tuning of LGN relay cells (McClurkin & Marrocco (1984)), in orientation sensitivity to patterns that go beyond the classical receptive field (Cudeiro & Sillito (1996), Sillito et al. (1993), Webb et al. (2002)), and in length tuning (Cleland et al. (1982), Jones et al. (2000), Murphy & Sillito (1987)). There is even a report that attention can affect processing in the human LGN (O'Connor et al. (2002)).

Przybyszewski et al. (2000) found that in the macaque, when only the classical receptive field is stimulated, the contrast gain of LGN cells is increased (except for magnocellular neurons at low contrast) by feedback from V1.

The early work of Tsumoto et al. (1978) suggests that cortical effects on LGN relay neurons depend, among other factors, on the topographic relationship between the interacting neurons: cortical neurons with receptive fields near that of an LGN neuron will be excitatory, while neurons with further receptive fields will exert inhibition.

Little work had been focused on how the dynamical properties of LGN receptive fields might depend on the corticofugal pathway. However, Funke & Eysel (1992) reported that under certain conditions (non slow wave sleep) the corticofugal pathway can have different impacts on the transient and sustained components of LGN discharge. In addition, Funke et al. (1996) reported that the cortical feedback improved the temporal precision of transmission from retina to LGN, and Sillito et al. (1994) reported that corticofugal feedback could, under the proper stimulus configuration, induce synchronization of LGN neurons in a way that could be used to link portions of an object.

Extraclassical receptive field effects: Felisberti & Derrington (1999) showed that, for relay
cells in anesthetized cats, sudden movements of gratings in the nearby periphery (annuli with radii of 5-15 deg) suppress the response to circular spots on their classical receptive field. The suppression increases with the contrast of the peripheral pattern. Retinal suppression appears to play a minor role, because the effect of the peripheral shift by itself is usually inhibitory on LGN cells, but excitatory on retinal ganglion cells. For extraclassical receptive field stimuli on a more local scale, Solomon et al. (2002) showed that responses are suppressed for all LGN cells in anesthetized marmosets. These results differ from what is observed when patterns move continuously (Mcllwain (1964)) or abruptly (Fischer & Kruger (1974, 1980)) in the far periphery of the classical receptive field, where the effect on both retinal ganglion and geniculate cells is excitatory.

Webb et al. (2002) showed that in anesthetized marmosets, during extraclassical receptive field stimulation, the role of V1 in LGN response is inhibitory, since V1 ablation leads to an overall increase in LGN relay cell response. Both orientation and location of stimuli beyond the classical receptive field modulate relay cell responses. These results were obtained by comparisons across animals, not from recordings of the same LGN cells before and after V1 ablation. They are in agreement with those obtained in cats (Sillito et al. (1993)), where it was shown that the corticofugal feedback is inhibitory and sensitive to stimulus orientation beyond the classical receptive field.

B.5.2 - Cortical Nonlinearities and feedback to the LGN

Cortical processing shows various forms of nonlinearity, which include nonlinear spatial summation (complex cells), context modulation (see above), rectification and direction selectivity. Several studies have investigated cortical nonlinearities in considerable depth (for example, Emerson et al. (1992), Gaska et al. (1994), Victor et al. (2004), among others). In order to understand fully the effects of the cortical feedback on the LGN, we must, therefore, examine both the linear and nonlinear aspects of LGN activity. Currently there is no information on the degree to which cortical nonlinearities affect LGN function. The use of the double-input m sequence (Benardete & Kaplan, 1997a,b, and see the METHODS section) will permit us to study these features.

B.5.3 - Brainstem input

Several studies have documented the modulatory effects of brainstem input on LGN function (Kayama et al. (1982, 1986a,b, 1989), Rogawski & Aghajanian (1980, 1982), Cucchiaro et al. (1988), Murphy et al. (1994), Uhlrich et al. (1988, 1990, 1995), Ozaki et al. (1997a)), which were expected because of the diffuse innervation of the LGN from various brainstem nuclei (Murphy et al. (1999), Uhlrich et al. (1988, 1990, 1995)). These effects are usually slow, and are thought to result from a modulation of the membrane potential of LGN relay cells. By raising or lowering the membrane potential, the T-Ca channel is activated or inactivated, and this conductance has a profound effect on the dynamics of the LGN relay neurons: when the membrane is hyperpolarized, the cell fires in bursts, but when it is depolarized, the firing is tonic (Llinás & Jahnsen (1982), Jahnsen & Llinás (1984a,b)).

A different type of (presumably) brainstem modulation has been reported in the primate by Reppas et al. (2002), who found that saccadic eye movements could alter significantly the response of LGN neurons to visual stimuli, and that the modulation depended on the nature of the stimulus.
B.5.4 - PGN input

The discharge of neurons in the perigeniculate nucleus (PGN) is anti-correlated with that of topographically connected LGN relay cells during visual stimulation (Funke & Eysel (1998)). The perigeniculate cells contribute to interocular inhibition, but for monocular stimuli their inhibitory influence on LGN relay cells cannot be distinguished from that of LGN interneurons (Funke & Eysel (1998)).

B.6 SIGNIFICANCE

What will our proposed experiments tell us?

The experiments proposed in this application will address a question not previously addressed, but which is crucial for our understanding of thalamic function: what role does the massive V1 → LGN feedback pathway play in shaping the dynamical and spatial properties of the LGN? Since it is known that, under some conditions, the temporal properties of LGN neurons differ markedly from those of the retinal ganglion cells that drive them, that difference must be due either to the biophysical properties of the thalamic neurons, or to the network in which they are embedded, or both. It has been known for some time that the temporal properties of LGN relay cells are not constant (for example, Kaplan et al. (1993)), and since the biophysical properties are not likely to change on a short time scale, it seems likely that the network exerts an influence on the dynamics. It is that influence which we seek to explore here. In addition, the information from the proposed experiments will be important and helpful for those who wish to construct biologically realistic computational models of the early visual system.

In brief, this project will explore the role of the cortical feedback in the following aspects of LGN function, most of which have never been examined before:

- Dynamics (linear and nonlinear)
- Receptive field spatial structure
- Transfer from retina to cortex
- gain
- Statistics of spike trains (probably at a later stage)

Functional Interpretations

The results of the proposed experiments should shed light not only on the consequences and possible mechanisms by which the cortex influences the LGN, but also on the purpose of that interaction. For instance, if we were to find that the effect of the feedback is mainly on the gain or transmission of retinal signals upstream, it might be related to directing attention to an important stimulus. If, on the other hand, we find significant alteration of either spatial or temporal properties of LGN receptive fields, this will point to cortical influence in perceptual processing, such as binding of spatial or temporal elements of the environment.

The proposed research will inform us, among other things, about the effect of the corticofugal feedback on the linearity of LGN responses. That, too, should help to determine the functional role of the feedback.
Clearly, the information that will be gathered is crucial for an understanding of the control of flow of visual information from retina to cortex.

C PRELIMINARY RESULTS and Relevant Studies

The studies described in this section document the fact that we have been actively involved in the study of the LGN (in both cats and primates) for a long time, and that we have much experience in all the experimental and analytical approaches that will be required in order to accomplish the Specific Aims of the proposed research.

Figure 2: An illustration of the S potential/Relay cell dual recording. Panel A shows what the two potentials look like. Panel B shows that following TTX injection into the eye, S potentials (and LGN spikes) disappear, proving that the only effective excitatory drive for the LGN relay neuron comes from the retina. Panel C shows the LGN spike rising from the S potential, and illustrates the increased variability of LGN spiking time compared with the less variable retinal firing (from Kaplan & Shapley (1984)).

C.1 The S potential: a tool for the study of transmission through the LGN

Over the years our laboratory has had much experience in the technique of recording synaptic ('S') potentials in the mammalian thalamus (Bishop (1953), Cleland et al. (1971), Kaplan & Shapley (1984)), which allows the simultaneous extraction of both retinal input (action potentials from retinal ganglion cells) and LGN output (Figure 2). The LGN output is recorded as conventional action potentials,
while the retinal input is recorded as slow ('S') potentials. Previous work has established that each retinal impulse is followed by an S potential, and we have shown in the past that retinal firing is the only source of S potentials in the LGN (Kaplan & Shapley (1984)), and therefore one may use them as a reliable monitor of retinal ganglion cell firing. We have made extensive use of this ability over the years, and have published several studies that were based on quantitative comparison of the retinal input with the LGN output (Kaplan & Shapley (1984), Kaplan et al. (1987), Levine et al. (1996), Lowen et al. (1998), Lowen et al. (2001), Teich et al. (1997), Mukherjee & Kaplan (1995), Mukherjee & Kaplan (1998)). Such quantitative comparisons allow us to focus on the transfer ratio (LGN spikes/retinal spikes) of an LGN neuron, and examine the factors that influence that transfer ratio. In addition, we have studied how activity in other brain centers affects the transmission of visual information through the LGN (Ozaki & Kaplan, submitted). Although we have shown that activity in the brainstem increases LGN transmission, we have not yet studied the effect on LGN dynamics.

C.2 Using multi m-sequence stimulation to study linear and non-linear dynamics in primate LGN (Benardete and Kaplan)

Various methods have been used to investigate the spatio-temporal properties, of visual neurons. Recently, the power of the m sequence and reverse correlation had been exploited by several groups for this purpose, and also for the investigation of chromatic and orientation selectivities of cortical neurons (see Reid et al. (1997), Ringach et al. (1997), Cottaris & DeValois (1998), Reid & Shapley (2002)).

Together with E. Benardete, the PI has studied the linear and nonlinear dynamical properties of ganglion cells in the primate retina (Benardete & Kaplan, 1997a,b; Benardete & Kaplan, 1999a,b; Kaplan & Benardete (2001)). These studies have demonstrated that in order to discover and analyze nonlinear properties, a special tool can be very helpful. The tool we used was the double m sequence stimulation method, in which two independent m sequences are added and used as stimuli, either for the center (spot) and surround (annulus) regions of a receptive field, or for two different cone classes that provide input to the receptive field. When used to study the center and surround, the results provide, in addition to the separate linear response kernels for the center and for the surround mechanisms, their second order kernels and the cross interaction (second order) term, as illustrated in Figure 3 (Benardete & Kaplan (1997b)).

C.3 Cortical inactivation

C.3.1 Inactivating V1 and studying LGN (Casti and Kaplan)

Recently, Casti & Kaplan (2004) have measured the temporal transfer functions of cat LGN relay neurons with and without feedback from V1. Some aspects of the results are illustrated in Figure 4, which shows that during cryogenic inactivation of the V1 feedback, the optimal temporal frequency is halved. The figure also shows a small but consistent phase advance as a result of inactivation of V1. Care was taken to ensure that the LGN was not affected directly by the cooling procedure. This example, and results from other cells, suggest that V1 indeed affects LGN dynamics, and that our cryogenic inactivation is effective.
Figure 3: Nonlinear responses (Second order kernels) from a primate parvocellular LGN cell, obtained with double m-sequence stimulation. Each row shows the response amplitude to 2 flashes presented at different times (shown on the x and y coordinates) as a wire-mesh plot (left) and as a contour plot (right). The first-order kernel was also measured from this response, but is not shown here. The center region of the RF was stimulated with a spot modulated by a double m sequence (top row), while the surround was stimulated by an annulus modulated by another, independent double m sequence (middle row). The bottom row shows the dynamics of the center-surround (cross terms) interaction (from Benardete & Kaplan (1997b)).
Figure 4: LGN Temporal Transfer Function with (red solid line, triangles) and without (blue dotted line, circles) V1 feedback. V1 was inactivated cryogenically. The stimulus was a drifting sine wave grating of optimal spatial frequency and 75% contrast.

C.3.2 Inactivating V2 and studying V1 (Sailstad and Kaplan)

Together with Cynthia Sailstad, we have investigated the effects of the feedback from cat V2 to V1 by reversible inactivation (by cooling or GABA injections) of V2, using optical imaging and electrophysiological recordings from V1. Figure 5 shows that V2 inactivation decreases the amplitude of the V1’s response to drifting (in this case, horizontal) gratings, and Figure 6 shows a decrease in the sharpness of orientation tuning as a result of feedback inactivation. In the course of carrying out these experiments, which revealed new features of the effects of the descending pathway from V2 to V1, we have gained the experience and mastery of the experimental techniques needed to perform the proposed research: cryogenic inactivation of the cortex, and targeted GABA injections into the cortex (Sailstad et al. (1999, 2000a,b, 2002)).
Figure 5: V2 inactivation lowers the amplitude of the intrinsic optical response to drifting gratings in cat V1. Activity Maps generated from images of V1 taken A) before, B) during, and C) after V2 was cooled (Sailstad & Kaplan, unpublished results).

Figure 6: V2 inactivation decreases the sharpness of orientation tuning in cat V1. Orientation bias maps calculated from V1 images before (A) during (B) and after (C) V2 inactivation. Sharply tuned regions are red; poorly tuned regions are blue. Orientation bias was calculated only for regions that responded above a predetermined threshold (Sailstad & Kaplan, unpublished results).
C.4  LGN modeling in our laboratory

In addition to experimental exploration of the LGN, our group has been engaged for several years in modeling the LGN, using several approaches. These studies, which include one by F. Hayot who has recently joined our group, are summarized briefly below.

C.4.1 A reduced LGN model. (Mukherjee and Kaplan)

Mukherjee and Kaplan (1995, 1998) have used the NEURON modeling program to construct a simplified model of the LGN relay neuron. The model had only 5 of the 11 ion channels that have been reported in the literature, yet it was able to capture the essence of the LGN dynamics. In particular, it exhibited the transition from tonic to bursty firing observed in experimental data (Mukherjee & Kaplan (1995, 1998)).

C.4.2 An extended LGN model. (Ozaki, Kaplan and Sirovich)

Following Mukherjee and Kaplan, Ozaki and Kaplan extended the LGN model to include all 11 types of ion channels that are known to exist in LGN relay cells. The extended model was able to account for experimental data from the LGN better than the previous, truncated model of Mukherjee & Kaplan (1995). The extended LGN model, with its 11 ionic channels, requires a 20 dimensional dynamical system for its description. Using techniques developed by members of our group (Sirovich et al. (1990)), we were able to find an adequate low dimensional description that captures the essential dynamics (Ozaki et al. (1996); Ozaki et al. (1997a); Ozaki et al. (1997b)). For simulations, these models lead to relatively complicated dynamical systems. Fortunately, it has been shown that, when simulating firing rate behavior, the general class of such Hodgkin-Huxley equations can often be well-approximated by the phenomenological integrate-and-fire equation (Kistler et al. (1997); Knight (2000)), which justify the use of integrate-and-fire dynamics in our simulations. When integrate-and-fire modeling is inadequate, other phenomenological models can usually be found, as illustrated in the next section.

C.4.3 Tonic/Bursty population model. (Casti, Knight and Sirovich)

Since relay cells can function in either a tonic or bursting mode, we built on the integrate and fire-or-burst model of Smith at al. (1998), and developed a population model of LGN neurons that captures both tonic and bursting behavior (Casti et al. (2002)). This model will be further modified and refined in the future. For example, since the activities of retinal ganglion cells and LGN neurons are highly correlated, the Casti et al. (2002) model will be coupled to an integrate-and-fire model of retina.

C.4.4 A model of corticothalamic feedback. (Hayot and Tranchina)

Hayot (who has recently joined our lab) and Tranchina (2001) constructed a model with retinal input into the LGN and feedback from layer 6 axons onto LGN relay cells. For simplicity, the inhibitory feedback due to interneurons and perigeniculate neurons went directly into relay cells. The signal transition through these inhibitory neurons was taken into account by an additional time delay, and by adding a circular symmetric blurring component to the inhibitory synaptic footprint, as compared to the excitatory one. The emphasis was on modeling the feedback from layer 6 cells, whose large,
orientation selective receptive fields were reported to be responsible for the sensitivity of LGN relay cells to orientation discontinuity beyond their classical receptive field (Sillito et al. (1993)). The model successfully described the experimental results for the stimuli considered, which showed that its implementation of the corticothalamic loop was adequate, despite a number of simplifications, such as the lack of explicit modeling of LGN interneurons and their retinal afferents.

That model suggested some of the features that could characterize feedback from V1, and is thus relevant to the current proposal. For instance, it showed that anisotropy of the feedback synaptic footprint is not of crucial importance, since isotropy can lead to similar results. It also showed that the various inhibitory effects observed in LGN relay cells, such as those related to length-tuning (Cleland et al. (1982), Jones et al. (2000), Murphy & Sillito (1987)), depend (for a given stimulus) on the elongated receptive fields of layer 6 cells. This will guide us in designing some of the stimuli we plan to use in the current project.

The model led to a number of testable predictions. One of these was that for a drifting grating stimulus outside a circular window centered on the receptive field center of an LGN relay cell, the response of the latter can be suppressed below its spontaneous firing rate if feedback is present, something which cannot happen when feedback is inactivated. This prediction was confirmed subsequently by Webb et al. (2002).

C.5 Publications from our lab that are relevant to the current proposal


D METHODS

We first describe the surgical preparation and experimental procedures that shall be used in the physiological experiments, and then describe the specific experiments and analysis procedures.

D.1 General experimental procedures

D.1.1 Surgical preparation

The experimental methods will be similar to those used in our laboratory in the past (Kaplan & Shapley (1982)). Adult macaque monkeys (Macaca fascicularis) will be anesthetized initially with an intramuscular (IM) injection of xylazine (Rompun, 2 mg/kg) and ketamine hydrochloride (Ketaset, 10 mg/kg), and then given propofol (diprivan) as needed during preparatory surgery. Local anesthetics (xylocaine) will be used profusely during surgery, and will also be used to infiltrate the areas around the ears. Anesthesia will be maintained with a mixture of propofol (4 mg/kg-hr) and sufentanil (0.05 µg/kg-hour), which will be given IV during the experiment. We have been using propofol and sufentanil for several years now, and because neither drug is a barbiturate, this mixture is a superior anesthetic/analgesic for work on the nervous system (see also Milne et al. (2003) for the benefits of using both drugs together). Furthermore, a recent report shows that propofol anesthesia causes no changes in blood flow in the occipital cortex (Fiset et al. (1999)). Cannulae will be inserted into the femoral veins, the right femoral artery, the bladder and the trachea. The animal will be mounted in a stereotaxic apparatus. Blood pressure, ECG, and body temperature will be measured and kept within the physiological range. Muscle paralysis will be produced by an infusion of vecuronium bromide (Norcuron, 0.25 mg/kg-hr), and the animal will be artificially respired. The respiration rate and stroke volume will be adjusted to produce an end-expiratory value of 3.5-4% CO2 at the exit of the tracheal cannula. Penicillin (750,000 units) and gentamicin sulfate (4 mg) will be administered IM to provide antibacterial coverage, and dexamethasone will be injected IV to prevent cerebral edema. A continuous IV flow (approximately 3 ml/kg-hr) of lactated Ringer’s solution with 5% dextrose will be maintained throughout the experiment to keep the animal properly hydrated, and the urinary catheter will monitor the overall fluid balance. Such preparations are usually stable in our laboratory for more than 96 hours. Observing the animal’s heart rate and blood pressure will help us to monitor the depth of anesthesia, and signs of distress, such as salivation or an increased heart rate, will be watched for. If any such signs appear, an additional dose of anesthetic will be administered immediately. Phenylephrine hydrochloride (10%) and atropine sulfate (1%) will be applied to the eyes. The corneas will be protected with plastic gas-permeable contact lenses, and a 3 mm diameter artificial pupil will be placed in front of each eye. The eyes will be refracted, and correcting lenses will focus the eyes for the viewing distance of the CRT monitor, usually 57 or 114 cm. The foveas will be back-projected and mapped with a modified fundus camera on a tangent screen, onto which the receptive field positions of single cells will be mapped.

The EEG and its power spectrum will be monitored continuously, as another indication of the depth of anesthesia, and to ensure that the cortex is as responsive and alert as possible, based on the results of Funke & Eysel (1992) who showed that the effect of cortical feedback depended on the state of the cortex as monitored by the EEG.
D.1.2 LGN and S potential recording

A craniotomy and durotomy will expose the brain over the LGN (H-C coordinate: 7A 11L) and the striate cortex (V1). The recording electrodes (insulated platinum-iridium) will be advanced towards the LGN in 1-micron steps with a stepping motor. By slowly bringing the electrode tip close to the cell body, it is possible to record both the S potential and the spike from the LGN relay cell (see figure 1). At the end of the recording from a given cell, the location of the recording electrodes will be marked with electrolytic lesions to allow the assignment of the recording sites to specific LGN layers. The time of occurrence of nerve impulses will be recorded to the nearest 0.1 msec during the experiment and stored by computer. When more than one neuron is recorded by the electrode, we shall perform off-line spike sorting, after which the spike trains will be binned, cycle-averaged and Fourier analyzed.

By the appropriate setting of the cutoff filters on our amplifiers, we shall record, together with spiking activity, the local field potential (LFP), which reflects the activity of many nearby cells.

Cell Classification: Each recorded LGN cell will be classified as a magnocellular or parvocellular neurons according to its conduction velocity, following the procedure we have used in the past (Kaplan & Shapley (1982)). A bipolar electrode will be embedded in the optic chiasm, and electric shocks (150 μsecond) will be delivered to the stimulating electrode, in order to measure their conduction velocity. The conduction velocity of magnocellular cells is significantly higher than that of parvocellular cells, and it will thus be easy to classify cells according to their conduction velocity.

D.1.3 Eye stabilization:

For the proposed experiments to succeed, it is important to prevent the eyes from moving during the measurement. This will be accomplished by gluing the eyes to stainless steel rings that are attached to the stereotactic frame. We use this procedure routinely, and it usually provides satisfactory stabilization. In addition, the position of a receptive field on the monitor screen will be checked periodically by an automated procedure that uses a bar, drifting back and forth first in the X and then in the Y direction, and uses the delay to the peaks of the averaged responses as an indicator of the receptive field position. If the eyes moved the stimulus position will be adjusted appropriately by the visual stimulating program.

D.1.4 Histology

After the experiment, the animal will be sacrificed with a bolus overdose injection of Nembutal, and perfused through the heart with saline, followed by 10% paraformaldehyde solution. The brain will be removed, fixed and sectioned, in order to reconstruct the electrodes' tracks.

D.1.5 Visual stimulation

Visual stimuli will be produced monocularly on a Sony CRT (mean luminance of 80-100 cd/m²) by a Cambridge VSG/2-5 stimulator. To ensure that the display is linear over the range of luminance levels used, it will be calibrated before each experiment with a Pritchard spectroradiometer (model 1980b), which has a built-in monochromator. Updated lookup tables will compensate for any drift in the luminance or linearity of the screen. Stimuli will be typically repeated at least 10-20 times in random order.
For spot and annulus stimulation (experiment #1), I shall start by measuring quickly the response of the LGN cell to drifting gratings of increasing spatial frequency past the cell's optimal spatial frequency, and determine the appropriate sizes of the center stimulating spot and center sparing annulus from the slope and intercept of the high frequency limb of the function (Linsenmeier et al. (1982)).

Other stimuli: To allow the examination of some other aspects of the LGN discharge (variability, spike timing and burstiness), I shall collect, for each cell, some data with no stimulation (0% contrast), and in response to contrast steps (10% and 50%) of a small spot centered on the receptive field.

The multi (double) m-sequence technique: We now described briefly the double m-sequence, which will be used to modulate our spot and annulus stimulus (experiment #1), and also the screen luminance in the cone isolating experiment (experiment #2). Further details can be found in Benardete & Victor (1994). An m-sequence, \( m(t) \), is a cyclic and binary signal that takes on a new value of +1 or -1 after a predetermined time step, \( T \) (Golomb (1968)). A positive value indicates an increment in contrast, while a negative value indicates a decrement. In our experiments, the contrast will be temporally modulated by a signal that is a sum of two m-sequences of relatively prime length. The use of pairs of m-sequences, chosen appropriately, prevents certain kinds of response contamination. With the usual (single) m-sequence method, determination of second- and higher-order kernels for multi-input systems is hindered by higher-order correlations (anomalies) that are intrinsic to the m-sequence method, and the double m-sequence technique effectively postpones these anomalies to third order.

In our experiments, the time step will be 14.8 ms. This modified m-sequence method allows a determination of first and second order kernels that describe how the cell being tested responds to its input. The first-order kernel assigns weights to successive stimulus steps of the m-sequence signal at their specific times in the past, according to how much each of those steps affects the current response. This kernel is similar to the impulse response of a linear system. To calculate the first-order m-sequence kernel, the response of the cell, \( r(t) \), is cross-correlated with the input sequence, \( m_1(t) \). The first-order response shows the temporal response to an impulse of contrast. The double m-sequence signal is generated from one sequence of length 31 and another of length 63. The total stimulus cycle is 1953 time steps, which is 28.90 seconds long.

The stimulation program inserts an additional 5 secs of stimulation at the beginning of each run. The response during this "pre-stimulus" epoch will be discarded, to avoid the transients in the response. The various stimulus conditions will be interleaved, and successive presentations of the same stimulus will use alternate m-sequences. This technique of using different component m-sequences and averaging the results also reduces anomalies in the kernel estimates (Benardete & Victor (1994)). The generating polynomials (Golomb (1968)) that determine the individual m-sequences used in successive sets are given in Benardete & Kaplan (1997a). The contrast of the central spot will be modulated by the sum of one 31- and one 63-length sequence, while the annulus will be modulated by long "lags" of the same two m-sequences. The spot and annulus will be modulated simultaneously in the receptive field of the LGN cell, to allow assessment of the center-surround interaction. The program will count the number of events (either LGN spikes or S-potentials) in each 3.7 ms "bin", and the response of the cell will be converted to spike rate (impulses/second). The cross-correlations necessary for kernel calculations will be computed through the use of the Fast M-Transform (FMT) described by Sutter (1992). A typical kernel calculation will incorporate approximately 3 minutes (6 "runs") or more of spike data. To avoid contamination from third order nonlinearities, the "inverse repeat" approach will be used (Benardete & Victor (1994)). Further technical details regarding the double m sequence method can be found in Benardete & Kaplan (1997b), and in
D.1.6 Inactivation of V1

We shall inactivate V1 (reversibly) with either of two methods: a) a global inactivation by cooling, or b) a localized, focused inactivation by GABA injections targeted into layer 6 of V1. We have performed control experiments to ensure that the inactivation is effective: recordings of multi-unit activity from a region under the cooling plate or near the GABA pipette have shown that we are, indeed, silencing the responses of these units. The effective range of our GABA inactivation is 0.5 mm, in agreement with published results (Hupé et al. (1998)).

The state of the cortex will be evaluated by examining the EEG recorded by a coarse electrode inserted under the cooling plate or near the injection site. The EEG power spectrum will be calculated on line. Its frequency distribution and overall signal amplitude are reliable indicators of the state of cortical activity: the EEG of an active cortex is dominated by high frequency, low amplitude fluctuations.

Details of the two main inactivation methods are described below.

1. Cooling

To inactivate V1, a 10×4 mm stainless steel cooling plate is placed on the exposed cortex, on the V1 region that represents the area near the fovea, and cold methanol is circulated through it. The cortex is then covered with silicon gel, which stabilizes the cooling plate and helps to keep the cooling effect localized to the area under the cooling plate, to prevent any direct cooling effect on other parts of the brain. Research by others (for example, Lomber et al. (1999)), which has been confirmed in our preliminary experiments, has shown that the full thickness of the cortex will become (reversibly) inactive when its temperature is lowered to approximately 9°C. The temperature of the cortex under the cooling plate is monitored with a thermistor. We also verified, by recording electrophysiologically from neurons under the cooling plate, that the cooling is, in fact, inactivating the cortex under the cooling plate.

2. Chemical inactivation

A refinement of the broad V1 cryogenic inactivation can be provided by silencing a smaller portion of V1, with a more precisely targeted inactivation of only layer 6 of V1, the layer from which the corticofugal pathway emanates. To achieve this goal, we shall use chemical inactivation by GABA, which will also permit inactivation of a region of V1 while leaving the rest to serve as a control. Approximately 2 μl of GABA (8 mg/ml) are sufficient to inactivate a significant portion of the cortex (Li et al. (1999)). We are following the procedures developed by Malpeli (1999), which have been tested successfully now in our laboratory. We use two micropipettes, 1 mm apart, and puff GABA from them with a picospritzer. We deliver 10 ms puffs at 20 psi, 15 puffs at 2 puffs/second, every minute for 45 minutes. Each puff delivers 13 nL of GABA. A recording metal electrode is carried by the same manipulator that also carries the GABA pipettes, and the depth of the electrode is marked by an electrolytic lesion, to ensure identification of the layer into which GABA had been injected. The recording electrode can also verify that when deep layers are injected, the superficial layers remain active. If it turns out that capillary action allows enough GABA to seep to the upper layers during deep layer injection, we shall angle the electrode as much as possible to minimize this effect.

In another inactivation technique we are currently testing, we place a piece of GABA-soaked Gelfoam on the cortex (Chatterjee & Callaway (2002)). Published results show that infusing the Gelfoam with GABA produces a complete inactivation of cortical activity, and our own preliminary results suggest that this procedure could be gentler than the cooling plate, with a better recovery.

D.1.7 A Complementary Approach: direct activation of layer 6

As a complementary approach to the study of the effects of V1 feedback, we shall, at the appropriate time, perform the following experiment, which follows closely the procedure used by Funke & Eysel (1992), where further details of the procedure are given. This experiment will increase (or decrease) the strength of the feedback from V1 to the LGN. A multi-barrel injection/recording electrode will be inserted into layer 6 of V1, which will contain (in separate barrels) GABA, quisqualate (quis, or in other experiments, NMDA) and saline. We shall perform our spatio-temporal mapping of the LGN receptive field, and then inject (iontophoretically) quis into layer 6, to increase the responsivity in a small patch of layer 6, and thus increase the feedback to the LGN, and then repeat the measurement. We shall then wash the quis away with saline, and inject GABA into the same area, thus reducing the V1 feedback in the same V1 patch, and repeat the spatio-temporal measurement a third time. It is uncertain that we shall be able to perform this experiment during the course of the funded period, but we include it here to indicate possible future directions for the study of V1 feedback.

D.2 Specific Experiments

The paradigm used in all the experiments will involve repeated measurements of the responses from an LGN relay cell (whenever possible, together with its retinal ganglion cell drive, recorded as an S potential). After a control measurement is obtained, V1 will be inactivated, and the measurement will be repeated. V1 will then be allowed to recover, and the measurement repeated one more time, to demonstrate recovery. When recording stability permits, the measurements will be performed at several contrasts, which span the operating range of the neuron. A blank screen (0% contrast) will always be included to provide an estimate of the inherent variability of the discharge.

Each measurement should take approximately 15 minutes, and the entire cycle of measurement → V1 inactivation → measurement → V1 recovery → measurement should take approximately 90-120 minutes. Our recording stability in the LGN is such that this is feasible, based on our years of experience of recordings in the cat and monkey LGN.

D.2.1 1: spot and annulus experiment

We shall use one double m sequence to modulate a small spot, centered on the receptive field of an LGN cell, and another double m sequence to modulate the surround at the same time. The size of the spot and annulus will be determined as described above. The measurement will be carried out before, during and after V1 inactivation.

Data analysis and interpretation: We shall reverse correlate the spike train with the double m sequence stimulus, to obtain the linear and second order temporal kernels of the LGN cell’s response, as was done by Benardete & Kaplan (1997a,b). The kernels obtained with and without V1 feedback will be compared, to determine which aspects of the dynamical response had been affected by the
descending cortical pathway: amplitude, contrast gain, phase lag, or various features of the second order kernel, including degree of rectification, and the extent, timing and strength of the center-surround interaction.

D.2.2 2: Cone isolating stimulation for 'type I' parvocellular neurons

This experiment will be performed only on 'type I' parvocellular cells, in which the center and surround of the receptive field appear to receive input exclusively from two distinct cone classes (M and L) (Wiesel & Hubel (1966), Reid & Shapley (1992, 2002)). We shall follow a procedure similar to that used by Reid and Shapley (1992), who used cone isolating stimuli to activate separately the center and surround mechanisms of parvocellular LGN neurons, but instead of using the pseudo random stimulus that Reid and Shapley used, we shall use the double m-sequence stimulus, in order to gain access to both linear and nonlinear components of the dynamical responses. One double m sequence will target the L cones, while the other will target the M cones. The details of the chromatic stimulus parameters are given in Benardete & Kaplan (1999a). As we described above in the spot/annulus experiment, we shall carry out the measurement before, during and after V1 inactivation. Spatially, the stimulus will be a uniform field modulated by the two double m sequences. This is somewhat similar to what was done by Cottaris & DeValois (1998), who studied the dynamics of V1 neurons in monkeys with a full field modulated with a single m sequence.

When recording stability permits, we shall (occasionally) perform both types of experiments (spot-annulus and cone isolation with full field) on the same parvocellular neuron, to compare the results from the two measurements.

Data analysis and interpretation: Because we use here a full field stimulus, we shall have only the dynamical response of the center and surround, both linear and nonlinear. However, in addition to providing this new information, this measurement will allow us to determine whether the cortical feedback influences the chromatic properties of LGN cells. This type of information is not currently available.

D.2.3 3: B/W m sequence checkerboard

For some magnocellular or parvocellular cells that are not 'type I', we shall use, in addition to the spot-annulus stimulus, a 16X16 checkerboard modulated by a single m sequence, similar to that used by Reid & Shapley (2002), but with black-white pixels, each modulated by an appropriately delayed version of the original m sequence. Since magnocellular receptive fields are larger than parvocellular ones, and since we want to detect activity that might extend beyond the classical receptive field surround, the size of the checkerboard on the retina will be adjusted to extend beyond the classical surround by moving the monitor closer to the eyes.

Data analysis and interpretation: Here again we shall reverse correlate the spike train with the temporal stimulus. This experiment will furnish not only dynamical information but the full spatio-temporal information for cells in which we cannot take advantage of the differential and specific center-surround cone connectivity. We shall look specifically in the spatial maps for 'extra-surround' or suppressive surround regions, which might be due to a more diffuse feedback connectivity.

For this experiment we choose a black and white stimulus, rather than cone isolating stimulus, in order to maximize the activation of the cell by exciting all the cone types.
D.2.4 4: Visual activation of layer 6 neurons

To increase the responses of layer 6 neurons in V1 (the ones that provide the feedback to the LGN), we shall use (before, during and after V1 inactivation) two types of visual stimuli:

1. Patches of drifting (2 Hz) gratings, arranged as shown in Figure 7.

2. Oriented stationary gratings, modulated by a double m sequence. The motivation for using these gratings is to include stimuli that were often used in other studies (for example, Benardete & Kaplan (1999a)), for comparison purposes, and also to increase further the probability of activating the cells in layer 6 of V1, beyond what might be expected from the shorter bars used in the stimulus illustrated in Figure 7 or in the checkerboard experiment.

The spatial frequency of the gratings for these stimuli will be chosen according to the retinal eccentricity of the LGN cell that is being studied, in accordance with our previous studies (Croner & Kaplan (1995)).

Data analysis and interpretation: The analysis of the drifting gratings responses will use the traditional Fourier analysis of the averaged PSTHs, and will compare the effect of V1 inactivation on the responses to the two stimuli (A and B) shown in Figure 7. When V1 feedback is inactivated, we would expect A and B to have an identical direct effect on the LGN cell, but to produce different responses under the influence of the feedback from V1, because we expect A to activate the orientationally tuned layer 6 neurons more vigorously.

The analysis for the stationary gratings will parallel the one for the first experiment, and the reverse correlation will furnish the temporal linear and nonlinear kernels of the cell’s responses.

Figure 7: An example of drifting grating patches arranged to form two stimuli that should stimulate the LGN and V1 differentially. Without V1 feedback, A and B should have the same effect on the LGN, but A should drive neurons in layer 6 of V1 more strongly than B, because layer 6 cells have elongated receptive fields tuned for orientation. The dashed circle around the central grating patch indicates the receptive field of the LGN neuron.
D.3 Further analysis and functional aspects:

The experiments described above will furnish new information about the following aspects of LGN function:

- **Spatio-Temporal maps**: Unlike previous attempts to elucidate the role of the V1 to LGN feedback, which focused primarily on (steady state) response amplitude or responsivity, our more complete measurements of both spatial and dynamical parameters of the receptive field are likely to uncover features that have eluded previous investigations.

- **Retina → LGN transfer ratio**: The simultaneous recording of the retinal input (in the form of S potentials) together with the LGN output, will provide an opportunity to assess the role that the corticofugal pathway might play in controlling the transfer ratio from retina to LGN. The excitatory and inhibitory inputs from V1 to the LGN could modulate the membrane potential of LGN relay cells, bringing them closer to (or away from) threshold, thus making it more or less likely that an incoming retinal ganglion cells spike will trigger an LGN spike. Therefore, after calculating the LGN and retinal kernels, we shall examine their ratio as a function of space and time. We recognize that maintaining a simultaneous recording for a long period of time may not always be successful, but the information about the retinal firing can be regarded as a free bonus, and is not essential for accomplishing the main goals of our project.

- **Transfer functions (frequency domain)**: The time domain kernels obtained from the experiments will be Fourier transformed into the frequency domain, in order to facilitate comparison with other published data and for model fitting at a later stage, as we have done in the past (Benardete & Kaplan (1997a,b; 1999a,b)).

- **Contrast dependence**: In a linear system, the output scales with input intensity, and measuring the response at one intensity is sufficient for predicting the response at other intensities. In a nonlinear system, however, one must investigate the entire range of relevant intensities, and that is why we propose to make our measurements at several intensities. Therefore, the results obtained at the various contrast levels will be compared. There is evidence that contrast affects various aspects of LGN response and transfer ratio (Kaplan et al. (1987), Cheng et al. (1995), Nolt et al. (2004)), so the comparison should be informative.

- **Linearity of response** The experiments will provide estimates of the linear and nonlinear components of the response. It would be of interest to see whether the cortical feedback can shift the LGN from a linear relay to a more nonlinear 'alarm bell'. The precise nature of the nonlinearity will also be informative, and will help to finger the cellular mechanisms that are involved.

- **Space-Time separability** An examination of the space-time maps that will emerge from the proposed experiments will allow us to determine to what extent space and time are separable (Dawis et al. (1984), Adelson & Bergen (1985), Cai et al., (1997)) in this system, and whether the cortical feedback affects this important property of the receptive field.

- **Burstiness** We shall also examine another measure of the LGN discharge that is related to, but not identical, to the preceding measure (linearity). Using the data from the contrast steps, we shall calculate the fraction of spikes that are contained in bursts (inter-spike interval < 4 msec) before
and during cortical inactivation. Our preliminary results (Casti & Kaplan (2004)) suggest that this fraction can be affected by the cortical feedback.

- **M, P and K channels and feedback from V1:** The significant differences between the various major neuronal channels in the primate LGN (M, P, K – see Kaplan (2003) for a recent review) have led to speculations about their distinct roles in visual processing. It is of interest, therefore, to know whether the feedback from V1 has the same or different effect on these quite different channels. Such observations could aid in understanding the role of the feedback, but they will also contribute to a better understanding of the functional roles of the M, P, and K channels. In particular, it would be interesting to determine whether the feedback affects the spatial aspects of the receptive field of parvocellular neurons (widely believed to be used in form, texture or color analysis), while for magnocellular neurons (reported to be important for motion or temporal analysis) the biggest effect will be found to be on their dynamics.

- **Local Field Potentials:** Analysis of these potentials (which will require only some more disk space, but no special measurement) will provide additional valuable information about the activity of LGN cells that are near the one from which we record spikes, and which are likely to be affected by the same descending cortical feedback that is impinging on the cell we are studying. Recall that Tsumoto et al. (1978) reported that the effect of layer 6 neurons on LGN relay cells depended on the distance between their receptive fields.

- **Spike timing, precision and statistics:** We shall subject the spike trains, initially, to several types of high resolution (narrow bin) timing analysis, including interval histograms and intervallograms (Wörgötter & Funke (1995)), to determine the extent to which the cortical feedback affects LGN spike timing and precision. If the results are encouraging, we shall apply to the data the type of metric analysis that Victor and his colleagues have developed (see, for example, Reich et al. (2000)), to determine to what extent the corticofugal pathway can influence the information content of the spike discharge. This analysis, which will be carried out at a later stage, will be done (with and without V1 feedback) for both spontaneous discharge and responses to steps of contrast.

### D.4 Possible concerns and pitfalls

- **Anesthesia:** In the proposed experiments, the animal will be anesthetized, and therefore the cortical feedback might not be able to exert its usual impact on the LGN. However, the anesthesia we typically use is propofol spiked with sufentanil. The combination allows us to use lower amounts than are required if used separately (see also Milne et al. (2003)). As noted above, propofol is thought to exert its main effect on the frontal lobes, so the occipital lobe should not be greatly affected. In addition, we are considering the use, at least in some experiments, of only sufentanil (without propofol). This anesthesia, which is often used in experiments on primate cortex, should rule out any effects of the propofol. Recall also that we are planning to monitor the EEG and its power spectrum, to keep the anesthesia from becoming too deep. Finally, our preliminary results (Figure 4), and those of others (Przybyszewski et al. (2000), Funke & Eysel (1992)) indicate that inactivation of V1 under anesthesia does have an effect on LGN responses.
• **Recording stability:** Because the experiments require a fairly extended period of recording from a single unit, we shall take special precautions to minimize brain movement. We shall suspend the animal to elevate the chest off the recording table (which is a pneumatic vibration isolation table), perform a pneumothorax, make the craniotomy as small as possible, and fill it with silicon gel or agar, to further reduce brain movement.

• **Adequate activation of layer 6 feedback neurons:** It is possible that the spot-annulus stimulus or the pseudo-random checkerboard will not stimulate optimally the neurons in layer 6 of V1, so the feedback effect will not be maximal. That is the reason I am planning to use the stationary oriented grating stimulus. Several orientations will be tried, to maximize the driving of the particular groups of V1 layer 6 cells that feed back onto the LGN cell I am studying.

• **Excitation and Inhibition:** The direct feedback to LGN from layer 6 of V1 is excitatory, while the feedback that arrives via the PGN is inhibitory. Inactivation of V1 will thus be expected to remove both excitation and inhibition, and might therefore not make a huge difference to the LGN. The positive preliminary results we present here (Figure 4) and the results of Przybyszewski et al. (2000) argue against this expectation. In addition, it is unlikely that the temporal characteristics of the direct and PGN mediated feedback paths will be identical, and since we shall be focusing on the dynamical aspects, we expect to find that the removal of V1 feedback will alter significantly the behavior of the LGN cells.

• **Comparison of responses of S potentials and LGN neurons to cone isolating stimuli:** If the responses of S potentials (proxies for retinal ganglion cells spikes) to these stimuli are not significantly different from those of LGN relay neurons, chances are that the V1 feedback has only a negligible effect on them, at least as seen from the perspective of these chromatic stimuli. However, this has never been tested rigorously, and in particular, has not been tested with a stimulus like the double m sequence stimulus that we are planning to use here. It remains, therefore, an open question. Since we believe that the cortical feedback subserves a useful function, we would expect its elimination to reduce the information content of the LGN discharge, or modify it in other ways.

**D.5 Time Table**

The various experiments and analysis will proceed simultaneously. However, we anticipate that, if the detailed examination of spike precision warrants it, analysis of spiking patterns using the spike metrics of [insert name] and his colleagues will be carried out at a later stage. We require 5 years to complete this study since the experiments are long and difficult, and each one will yield only a small number of neurons that could be analyzed.
E  HUMAN SUBJECTS

NA

F  VERTEBRATE ANIMALS

Species: Adult monkeys (Macaca fascicularis). Numbers: 10 monkeys will be used each year.
Rationale: We use monkeys because a great deal is known already about their visual system. In addition, their visual capabilities have been shown to be very close to those of humans (De Valois et al. (1974a,b,c)). We make every effort to study each animal for as long as possible (sometimes for 5 to 7 days). Nevertheless, we can perform only a limited number of experiments per animal, since each experiment lasts many minutes and sometimes hours. This requires that we use approximately 10 monkeys/year.
Maintenance: The animals are housed at a modern facility which meets all the provisions of the Animal Welfare Act and the NIH guidelines. The animals are under constant (and excellent) veterinary supervision.
Other details regarding the handling of the animals, euthanasia, as well as our extensive procedures used to ensure their freedom from pain and discomfort are described in the Methods section of this proposal. Our research is conducted within the NIH guidelines entitled: Responsibility for care and use of animals (Vol. 6, #17, 1978). The animals will be continuously monitored during the experiments for any sign of pain or distress, and corrective measures will be taken immediately should such signs (salivation, increased or irregular heart rate, increased blood pressure, etc.) be noted.
G LITERATURE CITED

References


H CONSORTIUM/CONTRACT ARRANGEMENTS

None

I CONSULTANTS

of Medicine, will serve as a consultant on this project (see attached letter). He was instrumental in the development of the double m-sequence method that I intend to employ in this project, and has had a long and productive interest in the analysis of neuronal dynamics. and I have collaborated and published together in the past.
CHECKLIST

TYPE OF APPLICATION (Check all that apply.)

- NEW application. (This application is being submitted to the PHS for the first time.)
- SBIR Phase I
- STTR Phase I
- REVISION of application number:
  (This application replaces a prior unfunded version of a new, competing continuation, or supplemental application.)
  
- COMPETING CONTINUATION of grant number: ________
  (This application is to extend a funded grant beyond its current project period.)
  Yes. If "Yes", mark previously reported.

- SUPPLEMENT to grant number:
  (This application is for additional funds to supplement a currently funded grant.)

- CHANGE of principal investigator/program director:
  Name of former principal investigator/program director:

- FOREIGN application or significant foreign component.

1. PROGRAM INCOME (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

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<th>Anticipated Amount</th>
<th>Source(s)</th>
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2. ASSURANCES/CERTIFICATIONS

The following assurances/certifications are made and verified by the signature of the Official Signing for Applicant Organization on the Face Page of the application. Descriptions of individual assurances/certifications are provided in Section III. If unable to certify compliance where applicable, provide an explanation and place it after this page.

- Human Subjects
- Research Using Human Pluripotent Stem Cells
- Research on Transplantation of Human Fetal Tissue
- Women and Minority Inclusion Policy
- Inclusion of Children Policy
- Vertebrate Animals
- Debarment and Suspension
- Drug-Free Workplace
- lobbying
- Non-Delinquency on Federal Debt
- Research Misconduct
- Civil Rights
- Handicapped Individuals
- Sex Discrimination
- Age Discrimination
- Recombinant DNA
- Financial Conflict of Interest
- STTR ONLY: Certification of Research Institution Participation.

3. FACILITIES AND ADMINISTRATION COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

- DHHS Agreement dated: 03/26/04
- No Indirect Costs Requested.

- No DHHS Agreement being negotiated with Regional Office
- No DHHS Agreement, but rate established with Regional Office

CALCULATION* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information. Supplying the following information on indirect costs is optional for for-profit organizations.)

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TOTAL F&A Costs $868,750

*Check appropriate box(es):
- Salary and wages base
- X Modified total direct cost base
- Other base (Explain)

Explanation (Attach separate sheet, if necessary.):

4. SMOKE-FREE WORKPLACE

- X Yes
- No (The response to this question has no impact on the review or funding of this application.)