NOTICE
The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

THÈSES CANADIENNES
La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopy de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE
Behavioral, anatomical and physiological studies of the geniculoso-suprachiasmatic tract in the golden hamster

by

Mary Harrington

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Dalhousie University, Halifax, Nova Scotia

© September, 1986
Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmter cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-03167-4
Dedication

This thesis is dedicated to Paul Cornwell, who advised undergraduate students to consider the job market and not to make a career out of physiological psychology, but who provided an example of a fun and interesting life that was impossible to resist.
# TABLE OF CONTENTS

Index to Figures and Tables vii

Abstract ix

Abbreviations xlv

Acknowledgments xlix

Chapter I. General introduction 1

Chapter II. Phase shifting behavior of hamsters with ablation of the geniculo-suprachiasmatic tract (GST).
   A. Introduction and literature review 9
   B. Experiment 1: Light pulse phase response curves 29
   C. Experiment 2: Dark pulse phase response curves 39
   D. Discussion 43

Chapter III. Characterization of neuropeptide Y immunoreactivity of and retinal input to GST neurons.
   A. Introduction and literature review 67
   B. Experiment 3: Neuropeptide Y immunoreactivity in GST neurons 87
   C. Experiment 4: Location of and retinal input to geniculate neurons immunoreactive to neuropeptide Y 99
Index to Figures and Tables

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
</tr>
<tr>
<td>9</td>
<td>65</td>
</tr>
<tr>
<td>10</td>
<td>109</td>
</tr>
<tr>
<td>11</td>
<td>111</td>
</tr>
<tr>
<td>12</td>
<td>115</td>
</tr>
<tr>
<td>13</td>
<td>117</td>
</tr>
<tr>
<td>14</td>
<td>119</td>
</tr>
<tr>
<td>15</td>
<td>121</td>
</tr>
<tr>
<td>16</td>
<td>123</td>
</tr>
<tr>
<td>17</td>
<td>127</td>
</tr>
<tr>
<td>18</td>
<td>127</td>
</tr>
<tr>
<td>19</td>
<td>177</td>
</tr>
<tr>
<td>20</td>
<td>179</td>
</tr>
<tr>
<td>21</td>
<td>181</td>
</tr>
<tr>
<td>22</td>
<td>183</td>
</tr>
<tr>
<td>23</td>
<td>185</td>
</tr>
<tr>
<td>24</td>
<td>187</td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

vii
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 26</td>
<td></td>
</tr>
<tr>
<td>Figure 27</td>
<td>191</td>
</tr>
<tr>
<td>Figure 28</td>
<td>208</td>
</tr>
<tr>
<td>Figure 29</td>
<td>249</td>
</tr>
<tr>
<td>Table 1</td>
<td>129</td>
</tr>
<tr>
<td>Table 2</td>
<td>131</td>
</tr>
<tr>
<td>Table 3</td>
<td>132</td>
</tr>
<tr>
<td>Table 4</td>
<td>135</td>
</tr>
<tr>
<td>Table 5</td>
<td>137</td>
</tr>
<tr>
<td>Table 6</td>
<td>138</td>
</tr>
<tr>
<td>Table 7</td>
<td>193</td>
</tr>
<tr>
<td>Table 8</td>
<td>195</td>
</tr>
<tr>
<td>Table 9</td>
<td>197</td>
</tr>
<tr>
<td>Table 10</td>
<td>198</td>
</tr>
</tbody>
</table>
Abstract

The putative neural pacemaker controlling circadian rhythms in mammals is contained in the suprachiasmatic nuclei of the hypothalamus. These nuclei receive a projection (the geniculo-suprachiasmatic tract or GST) from neurons in the lateral geniculate nuclei (LGN) of the thalamus. Several experiments were performed to characterize the GST and to assess its role in circadian organization.

Circadian rhythms of running wheel activity in golden hamsters (Mesocricetus auratus) with GST ablation differed from those of control hamsters. Phase advance shifts to both light and dark-pulses were reduced by ablation of the GST, while no differences were seen in phase delay shifts. Wheel-running rhythms of GST-ablated hamsters also failed to respond normally to housing under continuous illumination. Specifically, GST-ablated hamsters displayed rhythms with unusual periods and did not generate "split" activity rhythms typically observed in intact animals.

Anatomical experiments indicated by two methods that the GST is characterized by neuropeptide Y-immunoreactivity. First, neuropeptide Y-immunoreactivity was reduced in the suprachiasmatic nuclei after GST ablation; second, neurons of the GST were double-labeled for neuropeptide Y-immunoreactivity and for a retrograde tracer transported from the
suprachiasmatic nuclei. GST neurons were located in the intergeniculate leaflet (IGL) and parts of the ventral LGN (vLGN). These areas were shown to receive retinal input.

The responses of GST neurons to light were studied with extracellular electrophysiological recordings. Visual responses of neurons located in the IGL differed from those in the vLGN, with the great majority of IGL neurons showing sustained "on" responses, to diffuse retinal illumination. Discharge rates of these neurons increased monotonically as light intensity was increased and saturated over 2 - 3 log units of intensity changes. Many GST neurons had binocular input, and input from the ipsilateral eye was often inhibitory.

These results indicate that GST neurons can provide information about ambient light intensity to the suprachiasmatic nuclei. This input alters the responsiveness of the circadian system to photic information.
LIST OF ABBREVIATIONS

APP - avian pancreatic polypeptide
CT - circadian time
DD - constant darkness
dlLGN - dorsal lateral geniculate nucleus
GST - geniculo-suprachiasmatic tract
HRP - horseradish peroxidase
IGL - intergeniculate leaflet
LL - constant illumination
LD - light-dark cycle
n.s. - not significant; p > .05
PBS - phosphate buffered saline
PRC - phase response curve
REM - rapid eye movement
SCN - suprachiasmatic nucleus
s.e.m. - standard error of the mean
s.d. - standard deviation
TMB - tetramethyl benzidine
vLGN - ventral lateral geniculate nucleus
vLGNa to vLGNNe - divisions of the vLGN of the rat
vLGNee - external part of the external division of the vLGN
vLGNei - internal part of the external division of the vLGN
vLGNI - internal division of the vLGN

WGA-HRP - wheat germ agglutinin-horseradish peroxidase
ACKNOWLEDGEMENTS

I am indebted to many people for assistance with the research presented in this thesis. I'm afraid that I am not eloquent enough to properly thank Ben Rusak, my advisor and my friend. Ben provided every form of support and encouragement necessary for the completion of both this research and my education.

The anatomical studies presented here were made possible by the generous collaboration of Dwight Nance. Dwight was most generous with what is often the most limited resource, time. Cathy Legacy-Barrett, who assisted in many of the experiments in this thesis, was especially valuable for her perseverance when developing double-labeling techniques. Tim King and Frank Sasinek provided friendly photographic assistance. Jeff Radel helped with the morphometric comparisons. Nick Swindale helped during early experiments with horseradish peroxidase.

Physiology experiments were improved by assistance from Gerard Groos, Gordon Troop, Nick McGraw, Max Gynäder and Keith Grasse. Jim Enns and Ray Klein provided statistical advice. I am grateful to Donna Gougen, Bruno Losier and Patricia Dickson for technical assistance.
I would like to thank the Killam Foundation for their generous financial support during these four years. Thanks are also due to Vin LoLordo, who helped unravel several financial problems that arose.

Many people were kind enough to read various parts of this thesis and provide feedback. For this I would like to thank Steve Shaw, Dwight Nance, Joke Meijer, Pat Card, Bob Moore, three anonymous reviewers, and, of course, my committee members. Discussions with many people improved my ideas; I would like to especially thank Gail Eskes and Joke Meijer, who provided critical feedback second only to that continuously provided by Ben.

Gerard Groos, in the short time that I knew him, provided not only technical assistance and theoretical discussions, but also served as a model of a critical and demanding scientist with an endearing sense of humor. It is Ben, Gerard, Gail and Joke to whom I would like to give my final warmest thanks, for it was their influences that turned these years of "work" into play.
CHAPTER I. General introduction

Circadian rhythms, rhythms which have periods of about 24 h, are quite prevalent in biological systems. The adaptive value of such rhythms may be that they allow an organism to anticipate an event which occurs predictably on a 24 h basis. For example, an animal may have a better chance of survival if it ends its period of activity before a predator awakes, instead of running for cover only after catching sight of the predator. Some behavior patterns which vary over 24 h might be passively driven by external events; however, the use of the term "circadian" is reserved to connote rhythms which are endogenously generated. These rhythms will persist for at least several cycles in the absence of 24 h cues.

The hypothalamic suprachiasmatic nuclei (SNC) appear to be important in the control of mammalian circadian rhythms. This small group of densely packed cells is located slightly above the optic chiasm, near the midline in mammalian brains. Ablation of the SNC causes the loss of circadian rhythmicity in many physiological systems (Klein and Moore, 1979; Moore and Eichler, 1972; Van. den Pol and Powley, 1979; Stephan and Zucker, 1972; Stetson and Watson-Whitmyre, 1976; but see Prosser et
Several lines of evidence indicate that the SCN can act as a self-sustained oscillator. The SCN of many mammals show a daily rhythm in 2-deoxyglucose uptake (Flood and Gibbs, 1982; Schwartz and Gainer, 1977; Schwartz, Reppert, Eagen and Moore-Ede, 1983) and in rats this rhythm has been shown to persist in constant darkness (Schwartz, Davidson and Smith, 1980). A circadian rhythm in multiple unit activity has been shown to persist for up to 35 days in the SCN when it is neurally isolated from most of the rest of the brain in a so-called "hypothalamic island" (Inouye and Kawamura, 1979, 1982). Other brain areas, when surgically isolated from the SCN, show no circadian rhythm in multiple unit activity. A circadian rhythm in unit activity has also been demonstrated in cultured hypothalamic slices containing the SCN (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata, Oomura, Kita and Hattori, 1982). Rhythmicity can be restored after SCN ablation by grafting fetal SCN tissue into the third ventricle (Drucker-Colin et al., 1984; Sawaki et al., 1984). Strong evidence that the SCN can act as pacemaker for activity rhythms is provided by a study showing phase shifts in the onset of activity rhythms following electrical stimulation of the SCN in hamsters and rats (Rusak and Groos, 1982).
Light/dark cycles are probably the most important entraining signals to mammalian circadian systems under natural conditions. There are two known anatomical pathways by which information on retinal illumination may reach the SCN. Some retinal ganglion cells project directly to the SCN via the retinohypothalamic tract (see Figure 1A; Hendrickson et al., 1972; Mai and Junger, 1977; Moore, 1973; Moore and Lenn, 1972; Tigges and O'Steen, 1974). Retinal ganglion cells also project to the lateral geniculate area of the thalamus (Frost, So and Schneider, 1979; Hickey and Spear, 1976). Neurons in the geniculate in turn project to the SCN, via the geniculo-suprachiasmatic tract (GST; see Figure 1B). The area of termination of the GST in the SCN overlaps that of the retinohypothalamic tract fibers (Card and Moore, 1982; Legg, 1979a; Pickard, 1982; Kibak and Peters, 1975; Swanson, Cowan and Jones, 1974).

It is not at all clear just what functions are served by this indirect visual pathway from the geniculate to the SCN. As will be reviewed later, previous studies of entrainment capabilities of animals with geniculate lesions have failed to find dramatic effects attributable to the loss of this putative entrainment pathway. The aim of this thesis is to increase knowledge of the anatomy, physiology, and functional aspects of the GST. This work will hopefully
lead to a more thorough understanding of the functional role of the GST in the circadian system, and, ultimately, to a better understanding of the process by which light-dark cycles entrain mammalian circadian rhythms.

In the next chapter, I will describe experiments on hamsters in which the GST was ablated and circadian rhythms were monitored. Patterns of phase shifting to both dark pulses and light pulses were examined, and the phase response curves from animals with GST ablation were compared with those of control animals. The light pulse phase response curve can be used to describe how an oscillator might maintain entrainment with a light-dark cycle (Pittendrigh and Daan, 1976a; see Appendix C). It is thought that these phase response curve measures may provide a better analytical tool for understanding changes in entrainment mechanisms after GST ablation than the cruder measures of previous studies.

The changes observed in phase shift responses described in chapter II could be related to phase shifts observed in hamsters after microinjections of neuropeptide Y into the SCN (Albers and Ferris, 1984). In chapter III, anatomical experiments designed to characterize the relationship between neuropeptide Y and the GST are described. Double-labeling and lesion
studies established that neuropeptide Y immunoreactivity is associated with the GST. Further experiments defined the location of neuropeptide Y-immunoreactive neurons in the geniculate area and the patterns of retinal input to these neurons.

From the patterns of retinal input to GST neurons, it was hypothesized that these neurons might have visual responses different from those of other neurons in the geniculate area. In chapter IV, results from extracellular recordings of single unit activity in the geniculate area are presented. In these experiments, responses of GST neurons to whole eye illumination were characterized and compared with those of neurons in surrounding areas. Luminance thresholds were measured and compared with those of visually responsive SUN neurons.

The final chapter summarizes the major results of these experiments: Electrophysiological studies of GST neurons suggested that GST neurons provide information about light intensity to the pacemaker neurons of the SCN. When these results were related to a quantitative model of a circadian pacemaker such as that proposed by Enright (1980), they suggested that effects of GST ablation might be modeled by changing the dynamics of the variable in this model (the threshold) which is posited to change with light intensity. This approach
proved fruitful, both in predicting the behavioral effects of GST ablation described in chapter II and in predicting further effects of GST ablation which have not yet been described and which provide future tests of this model.
Figure 1. A schematic representation of putative photic entrainment pathways in the hamster brain. Retinal ganglion cells send axons in the optic nerve (ON) to the area of the optic chiasm (OC). Some axons terminate bilaterally in the suprachiasmatic nucleus (SCN) ventral to the third ventricle (V III) (see hatched area in A.). Other retinal ganglion cell axons project past the OC and follow the optic tract (OT) to terminate in the lateral geniculate nucleus (LGN). Some neurons in the LGN send axons along the geniculo-suprachiasmatic tract (GST) to the SCN (see dotted area in B.). Three frontal sections of the brain are represented in B., arranged rostral to caudal.
CHAPTER II. Phase shifting behavior of hamsters with ablation of the geniculo-suprachiasmatic tract (GST).

II.A. Introduction and literature review

In this chapter, experiments testing the phase shift responses of hamsters with geniculo-suprachiasmatic tract (GST) ablation will be described. The destruction of the neurons of origin of the GST entails destruction of most of the ventral lateral geniculate nucleus. In the first section of the introduction, I will review previous work related to effects of ventral lateral geniculate nucleus ablation which may be additional to effects of such ablation on the circadian system. In the second section, I will review previous studies of effects of GST ablation on circadian rhythms. The third section will describe the rationale for the experiments to follow.

II.A.1. Visual discrimination deficits after ventral lateral geniculate ablation

Several studies have examined the effects of ventral lateral geniculate nucleus (vLGN) lesions on general visual capabilities. An unambiguous role for the vLGN in visual functions has not yet been determined;
although perhaps the most consistent finding is a deficit in visual intensity discrimination after vLGN ablation. One difficulty in interpreting the studies below is that vLGN ablation is usually confounded with incidental destruction of the primary optic tract and the dorsal lateral geniculate nucleus, due to the proximity of these structures to the vLGN. Control animals with only dorsal lateral geniculate and primary optic tract damage are required for firm conclusions about visual deficits attributable specifically to the vLGN.

Horel (1968b) studied the effect of large vLGN lesions on a black-white discrimination task using rats. Rats with vLGN ablation required more trials to relearn the task than was required to learn it before surgery. The total size of the lesion was highly correlated with the amount of deficit observed; however, the amount of optic tract damage was as strongly correlated with the amount of deficit as was the amount of damage specific to the vLGN, indicating that the lesion effects may have been due to incidental optic tract damage. Horel (1968a) also reported that knife cuts medial to the vLGN that extended into the zona incerta prevented retention of a previously learned black-white discrimination. The tentative conclusion of the author is that the vLGN is involved in relaying information concerning the density
of luminous flux, but these data are not very convincing due to the lack of proper controls.

Thompson, Truax and Thorne (1970) studied the effects of knife cuts between the vLGN and the posterior nucleus of the thalamus on retention of a visual discrimination. Rats with knife cuts which completely severed the connections between the vLGN and the posterior nucleus failed to relearn a discrimination between a large and a small white disk. Animals with knife cuts erring in the rostro-caudal plane so that the transection between these two nuclei was incomplete were indistinguishable from controls in their retention of previously taught discriminations. From these results, it seems possible that a connection between the vLGN and the posterior thalamus is essential for the formation and maintenance of some visual discriminations in the rat.

Legg (1975) examined the pupillary light reflex in rats with various subcortical lesions. The pupillary light reflex has long been thought to be controlled in part by the pretectal thalamic nuclei. Legg found that half of the rats with vLGN lesions showed mydriasis (abnormally dilated pupils). These rats had large lesions that damaged most of the vLGN bilaterally. The animals with vLGN lesions could perform a visual pattern discrimination task. Legg took this to indicate that
the optic tract was not wholly severed and that the mydriasis seen after vLGN lesions was not simply due to destruction of the fibers from the retina to the pretectal nuclei. There was no explanation for why some rats sustaining large vLGN lesions did not show mydriasis.

Legg concluded that the vLGN is involved in the control of the pupillary light reflex. This conclusion is weakened by results of a study by Schneider and Jhaveri (1983). Using knife cuts of the optic tract fibers running along the lateral edge of the vLGN, Schneider et al. showed that the optic tract fibers that course through the main body of the vLGN project specifically to the pretectum. Thus, the effects of vLGN lesions observed by Legg could very well be due to destruction of the retinal input to the pretectum, even if it appeared that retinal input to other thalamic nuclei was functionally intact.

Legg and Cowey (1977a) reported a series of experiments indicating that the vLGN may play a role in light intensity discriminations. They assessed capabilities of rats with vLGN lesions in the performance of either black-white (intensity) or vertical-horizontal (orientation) discriminations. In the first experiment, postoperative acquisition of these two tasks was assessed in animals with various
subcortical lesions. Rats with lesions of the vLGN or the posterior thalamus were impaired on the intensity but not the orientation discrimination when compared to sham-operated controls. In the second experiment, animals received more discrete vLGN lesions and postoperative acquisition of the two tasks was again assessed. Damage to the vLGN varied between 35 and 90% and damage to the surrounding visually-related nuclei was minimal. The same pattern of results as previously described was observed. In the third experiment, the same tasks were used, but postoperative retention was assessed. The vLGN-lesion animals were again impaired on the intensity but not the orientation discrimination as compared with sham-operated controls.

Legg and Cowey (1977b) carried this research further with different tests of light intensity discrimination. They found that light avoidance was unimpaired after vLGN lesions in rats. The previous finding, that rats with vLGN lesions were impaired on an intensity but not an orientation discrimination, was replicated. Further work indicated that this impairment was seen whether stimuli were presented simultaneously or successively. In the final experiment, brightness discrimination thresholds were assessed. Results suggested that the rats with vLGN lesions had elevated relative brightness thresholds, although absolute thresholds were the same.
as controls. These findings arose from data on a total of 5 rats with vLGN lesions and 6 sham-operated rats. All 5 rats had bilateral damage (ranging from 35 to 90%) to the vLGN. The variability in extent of damage to the vLGN and the small number of animals tested severely limits the conclusions that may be drawn from this study.

Legg (1979b) reported that animals with lesions in the lateral and posterior portions of the zona incerta were impaired on black-white and bright-dim discriminations but not on horizontal-vertical discriminations. Since this pattern of deficits is similar to that previously reported following vLGN lesions, it is possible that visual intensity information is relayed from the vLGN to other parts of the nervous system via the zona incerta.

In summary, it appears that the most common deficit in visual discrimination observed after vLGN ablation is one of discrimination of light intensity levels. This deficit may be due to ablation of vLGN neurons or, alternatively, it may be due to destruction of retinal fibers or of nearby thalamic nuclei. Better controlled studies, possibly making use of neurotoxins which selectively destroy neurons and not axons, will be required before a functional role for the vLGN in visual perception is defined.
II.A.2. Changes in circadian rhythms after disruption of the ventral lateral geniculate nucleus

Studies of the role of the vLGN in circadian rhythms have utilized animals with destruction of either the vLGN or the primary optic tracts posterior to the chiasm. Ablation of the primary optic tracts would involve destruction of primary visual afferents to the vLGN (as well as those to the rest of the thalamus and the tectum), but would presumably leave the retinohypothalamic tract to the SCN intact. In addition, depending on the placement, primary optic tract lesions may damage neurons in the vLGN or the lateral hypothalamic area. The lateral hypothalamic area receives direct retinal innervation (Kita and Oomura, 1982; Mai, 1979; Riley, Card and Moore, 1981) and has been suggested as the locus for the oscillator controlling rhythms entrained by food and water restriction (Riley, Card and Moore, 1981; but see Mistlberger and Rusak, 1985). Lesions of the vLGN or the primary optic tract would also probably destroy at least some of the dorsal raphe afferents to the SCN (Azmitia and Segal, 1978). Some raphe cells have been shown to be responsive to visual stimuli (Mosko and Jacobs, 1974). Thus, changes in the responsiveness of
circadian rhythms to lighting conditions following primary optic tract lesions may not in every case be attributable to the loss of retinal innervation to the vLGN. Both the lateral hypothalamic area and the dorsal raphe are areas that may possibly provide photic information to the circadian system.

Primary optic tract lesions do not appear to disrupt daily rhythms of pineal hydroxyindole-0-methyltransferase activity (Chase, Seiden and Moore, 1969), adrenal corticosterone levels (Moore and Eichler, 1972), or pineal N-acetyltransferase activity (Klein and Moore, 1979).

Stephan and Zucker (1972) studied the effects of primary optic tract lesions on circadian rhythms of water intake. In this study rats received large primary optic tract lesions, including extensive damage to the LGN and to surrounding areas. Under a light:dark (LD) cycle, these animals resembled intact rats (Zucker, 1971) in that they confined approximately 90% of their water intake to the dark phase. Under continuous illumination, rats with primary optic tract lesions showed an enhanced suppression of water intake (measured relative to post-operative baseline measures of water intake under an LD cycle) as compared with pre-operative measures. The rate of acquisition of nocturnality following reversal of the LD cycle was also measured.
An informal comparison of rats with primary optic tract lesions to intact rats from another experiment indicated that by the third day the two groups did not differ in their degree of nocturnality, although in the first 2 days the primary optic tract-lesion animals were phase-advanced relative to the intact rats. The authors concluded that primary optic tract lesions do not significantly affect photic entrainment of drinking rhythms. However, the measure of drinking rhythms used in this study (% of water intake in the dark phase) would probably not be sensitive to subtle changes in entrainment patterns. As well, a proper control group of intact or sham-operated rats was not included in this study.

Zucker, Rusak and King (1976) studied the effects of large LGN lesions on the circadian rhythm of wheel running in golden hamsters. Wheel-running patterns of both control hamsters and hamsters with bilateral or unilateral LGN lesions were exclusively nocturnal during maintenance on a 12:12 LD cycle. The rate at which wheel running re-entrained following a reversal of the LD cycle was slowed in hamsters with LGN lesions. The control hamsters required a range of 5 to 13 days to achieve a new stable phase angle, as compared to the hamsters with bilateral LGN ablation which required 22 to 30 days to accomplish the same phase shift. The
hamsters with unilateral LGN lesions did not appear to differ from control hamsters. The authors concluded that the primary optic tract may be of considerable importance for responding to phase shifts in the illumination cycle, perhaps because of disruption of the projection from the LGN to the SCN. However, conclusions from this study are limited by the very small number of animals tested (a total of 12). This is especially critical in light of Stephan and Zucker's (1972) indication that rats with primary optic tract lesions do not differ from intact rats in their rate of re-establishing nocturnality of drinking rhythms following LD cycle reversal.

In a later study (Rusak, 1977) photic entrainment of wheel running activity was studied in golden hamsters which had received primary optic tract lesions that did not damage the LGN. Five hamsters had complete bilateral destruction of the primary optic tract. One of these animals showed normal entrainment and a rhythm with a normal period. The other four animals demonstrated some abnormalities in the pattern of their activity rhythms. The most consistent difference found between these animals and sham-operated controls was that of unusually long periods of their free-running rhythms in dim constant illumination. Two of these hamsters phase-shifted very slowly to a 4 h phase delay
in the LD 14:10 lighting cycle. These 2 hamsters also showed an unusual phase angle of entrainment to the LD cycle, extending their activity into the late light phase. Since one animal sustaining a primary optic tract lesion did not differ from the controls, one can conclude that the primary optic tract is not necessary for stable entrainment in hamsters. However, these data may indicate a possible modulating role of the primary optic tract, affecting period of activity rhythms in constant light and possibly also affecting entrainment patterns.

This issue was re-examined by Rusak and Boulos (1981) using hamsters with lesions of the primary optic tract similar to those in the previous study. Hamsters were maintained under either LD, constant light or constant darkness prior to and immediately after surgery. Some animals were later transferred to one or both of the other lighting conditions. Under all three lighting conditions, changes in the pattern of activity rhythms were attributable to the lesions, but these effects were quite variable between animals. Under an LD cycle, some animals showed an increase in the duration of their active phase. Most of the hamsters that received lesions while in constant darkness showed an increase in the period of their rhythm within a few days of surgery, usually accompanied by a lengthening of
the duration of the active phase. Under constant light, some animals showed a dramatic lengthening of their rhythm's period. Others, however, showed a temporary decrease in period after the lesion, which then increased again after several days or weeks, stabilizing at pre-lesion values. An effect of the lesions which seemed to be unrelated to the lighting condition was an increase in total wheel-running activity seen in some animals. It was suggested that the effects of primary optic tract lesions observed in this and the previous study may be due to changes in coupling between two or more oscillators controlling activity rhythms.

Donaldson and Stephan (1982) studied the effects of LGN lesions on circadian organization of drinking rhythms in rats. Since the major purpose of this study was to examine effects of altered symmetry of visual input to the SCN, these lesions were combined with unilateral SCN lesions and with unilateral enucleation in some cases. Animals were first tested under a LD 12:12 cycle. This lighting cycle was then reversed. Three weeks later animals were housed in total darkness and then in constant light, each for 20 days. No differences between intact rats and rats with LGN lesions were noted under any lighting cycles. These rats were then given the same series of lighting conditions after unilateral eye enucleation. Both
groups required significantly more days to re-entrain to the reversed LD cycle after unilateral blinding than they did with both eyes intact. Therefore, it appears that rats with LGN lesions respond to altered symmetry of visual input in a manner similar to intact controls. This may indicate that effects of unilateral blinding can be mediated by altered SCN, as opposed to LGN visual input. The decreased rate of re-entrainment after unilateral blinding probably cannot be explained by a simple decrease in amount of visual input to the SCN, since rats with LGN lesions and unilateral enucleation might then be expected to show a rate even slower than that of rats with unilateral enucleation alone. Donaldson and Stephan favor the hypothesis that altered symmetry of visual input to the SCN is responsible for changes in the rate of re-entrainment. However, Zucker, Rusak and King (1976) reported that the rate of re-entrainment of hamsters with unilateral LGN lesions was faster than that of hamsters with bilateral LGN lesions which presumably left the SCN with symmetric visual input. Thus, at this point it is unclear what importance symmetry of visual input may have for the circadian system. It does appear that LGN lesions do not affect the rate of re-entrainment of drinking rhythms to a reversed LD cycle in rats. This indicates the need for replication of the study (Zucker, Rusak and
King, 1976) showing the opposite result using wheel-running rhythms of hamsters.

Sisk and Stephan (1982) examined sleep patterns of rats with primary optic tract lesions housed under either 24 h (LD 10:14) or 1 h (LD 0.5:0.5) lighting cycles. This experiment was run in four consecutive days. During the first 2 days the LD 10:14 cycle was presented and during the last 2 days the LD 0.5:0.5 condition was imposed. Differences in the distribution of rapid eye movement (REM) sleep between the group of rats with primary optic tract lesions and the intact control rats were observed under both lighting cycles. Under LD 10:14, the control group rats showed REM sleep evenly distributed throughout 24 h. In the group of rats with primary optic tract lesions, time spent in REM sleep decreased to very low levels in the last half of the dark phase. This could indicate that the primary optic tract normally plays a role in inhibiting circadian modulation of REM sleep distribution. Under LD 0.5:0.5, the control rats confined almost all of their REM sleep to the dark phases. The rats with primary optic tract lesions, on the other hand, showed only slightly more REM in the dark periods than in the light periods. This result suggests that the primary optic tract plays a role mediating direct (i.e., "masking") effects of light on REM sleep distribution.
Interpretation of these data is difficult due to the fact that data from all animals in a given condition were grouped for analysis. It is not clear that individual animals showed the pattern seen in the grouped data. In addition, since it is possible that animals with primary optic tract lesions may show deficits in entrainment to lighting conditions, more than 2 days should probably have been allowed for entrainment to the LD cycles used in this study. However, the suggestion from this study that rats with primary optic tract lesions show REM sleep patterns differing from those of intact rats should be examined further and animals with LGN lesions should be tested for similar deficits.

In conclusion, it appears that neither primary optic tract (Stephan and Zucker, 1972) nor LGN (Donaldson and Stephan, 1982) lesions alter photic entrainment of drinking rhythms in rats. Although it has been reported that hamsters with LGN lesions show slower re-entrainment of activity rhythms to a reversed LD cycle (Zucker, Rusak, and King, 1976), replication of this finding is necessary in light of the absence of a similar effect found for drinking rhythms in the rat. After primary optic tract lesions, hamsters show various irregularities in activity rhythms, the most consistent of which is an increased period; however, these effects
are quite variable across animals (Rusak, 1977; Rusak and Boulos, 1981). Finally, there is some evidence that primary optic tract lesions in rats lead to a change in the distribution of REM sleep under various lighting cycles (Sisk and Stephan, 1982).

A problem common to all of these studies is that the lesions were histologically assessed without the aid of modern anatomical techniques. Thus, lesions of the primary optic tracts could have been assessed after injections of anterograde tracers into the eyes produced labeling of surviving retinal afferents. As is discussed in chapter III, the density of neuropeptide Y-immunoreactive fibers in the SCN provides an estimate of the degree of damage to the GST. Since the intent of these studies was to destroy the GST and not the LGN per se, this would be a useful way to assess the lesions.

II.A.3. Introduction to phase response curve experiments

The failure of previous studies to demonstrate a clear role for the vLGN in circadian organization may be due to insensitivity of the assays used to measure changes in the entrainment mechanism. Alternatively, rodents may be able to entrain circadian rhythms using either the projection from the geniculate to the SCN or
the projection from the retina to the SCN. It is technically very difficult to selectively destroy the retinohypothalamic tract, sparing the retinal input to the vLGN and the projection from the geniculate to the SCN. Thus, it has not been possible to use lesions of the retinohypothalamic tract to identify separate contributions of the retinohypothalamic tract and geniculo-suprachiasmatic tract to entrainment of circadian rhythms. It may be possible, however, to determine if animals with GST ablation differ from control animals in their entrainment capabilities simply by using behavioral assays which are potentially more sensitive than those utilized previously.

One behavioral assay which may be more sensitive than those used previously is the phase response curve (PRC). The PRC is constructed by measuring the phase shifts in rhythms induced by treatments given at various times of the day relative to a standard phase of the rhythm (a "circadian time"). For hamster activity rhythms, activity onset is generally used as the standard phase and is labeled circadian time (CT) 12. Since a hamster's activity normally occurs during the dark part of a light-dark cycle when the hamster is housed under constant lighting conditions we call the half of the cycle in which the hamster is active the "subjective night". The 'other' half of the cycle is
called the "subjective day". Phase response curves for light pulses given to hamsters housed in constant darkness show phase delays in the late subjective day and early subjective night (CTs 10 to 14) and phase advances in the late subjective night (CTs 18 to 22). Pulses given through most of the subjective day have little or no phase shifting effect. This pattern of response has been incorporated into a theory of entrainment that accounts for many aspects of circadian organization (Fittendrigh and Daan, 1976b; see Appendix C).

In addition to the light pulse PRC, it is also possible to construct a PRC for dark pulses given to animals housed in constant light. The dark pulses must be considerably longer than light pulses to elicit a significant phase shifting effect (e.g., 6 h vs. 15 min). The PRC thus generated differs from that for light pulses. Dark pulses in the subjective day and early subjective night advance the rhythm, while pulses in the late subjective night produce phase delays. There appears to be no time of day when pulses have no effect. The determination of the dark pulse PRC has been relatively recent (Boulos and Rusak, 1982; Ellis, Mclveen, and Turek, 1982) and has been difficult to incorporate into previous theories of entrainment. The dark pulse PRC is of special interest here, however, due
to a report by Albers and Ferris (1984) on the pattern of phase shifts after microinjection of neuropeptide Y near the SCN of a hamster. These phase shifts varied in direction and magnitude with the phase of the animal's rhythm at which the neuropeptide Y was administered. The resulting PRC closely resembled the PRC for dark pulses. At least some of the cells of the GST may be identified by their immunoreactivity to neuropeptide Y in the rat and hamster (Card and Moore, 1982; Harrington, Nance and Rusak, 1985). Thus, Albers' results may indicate that the GST is involved in producing phase shifts to dark pulses, perhaps by release of neuropeptide Y. In support of this hypothesis, a similar phase response curve was observed for hamster activity rhythms after electrical stimulation of the vLGN (Meijer, Rusak and Harrington, 1984).

The experiments to follow were designed to assess phase response curves for both dark pulses and light pulses after GST ablation in hamsters. A decrease in advancing phase shifts in the GST-ablated animals was observed in the PRCs for both types of pulses. GST-ablation did not appear to alter delaying phase shifts. Animals with GST ablation also differed from controls in the responses of their activity rhythms to exposure to constant light; their rhythms had shorter
periods during continuous illumination and they were less likely to show rhythms split into two components coupled in approximate antiphase.
II.B. Experiment 1: Light pulse phase response curves

II.B.1.a. General methods for Experiments 1 and 2

Adult male golden hamsters (Charles River Lakeview) were taken from a colony room kept under a 14:10 h light dark cycle (lights on 0600 h to 2000 h, Atlantic Daylight Time) and were housed in individual cages (46 X 25 X 20 cm). Cages were cleaned and food and water replenished approximately every ten days at random times during the light phase. A dim (3 lux) red flashlight was used if hamsters were housed in constant dark; no phase shifts were ever observed in response to routine exposure to this light. Cages were equipped with running wheels (17 cm in diameter) whose rotations activated a pen on an Esterline Angus event recorder. Each individual hamster's running wheel record was cut into segments representing 24 h and each day's record was pasted below the previous day's.

After freerunning rhythms were established, hamsters were exposed to light or dark pulses given at various circadian times (CTs), with CT12 being defined as onset of running-wheel activity and all measures normalized to correct for differences in freerunning periods (Pittendrigh and Daan, 1976a). A minimum of 10 days separated pulses to allow stabilization of the
rhythm. Phase shifts were assessed by calculating two regression lines, one line through at least ten successive activity onsets before the pulse and the other line through ten onsets after transient responses to the pulse had disappeared. These regression lines were extrapolated to the first day after the pulse, and the difference between the two extrapolated onset phases was used as an estimate of the phase shift.

Some hamsters received radiofrequency lesions intended to ablate bilaterally both the intergeniculate leaflet (IGL) and the anterior vLGN, areas which contain cells which project to the SCN (see chapter III; Pickard, 1982). Surgery was performed under sodium pentobarbital anesthesia (80 mg/kg). Hamsters were positioned in a Kopf stereotaxic instrument with the incisor bar 2.0 mm below the interaural line. Radiofrequency lesions were placed at three rostrocaudal positions (1.6, 1.1, and 0.6 mm caudal to bregma; 3.0, 3.15, and 3.2 mm lateral to the midline; and 5.0, 4.65, and 4.4 mm ventral to dura) using a Grass LM4 lesion maker. Current equivalent to 12 - 15 mA DC was passed for 15 seconds through an electrode constructed from a stainless steel insect pin insulated except for 0.5 mm at the tip. Sham-operated hamsters (n = 20) were treated exactly the same as hamsters receiving lesions except that electrodes were lowered to 1 mm above the
positions where in other animals lesions were created and no current was passed. Animals that died or showed poor running records were replaced by new hamsters; a total of 62 operated hamsters were studied.

At the end of both experiments hamsters with lesions were given a lethal dose of sodium pentobarbital and were perfused with an intracardiac rinse of 1% buffered sodium nitrite, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2 - 7.4). Tissue was processed for neuropeptide Y-immunoreactivity in the SCN using previously published techniques (Harrington, Nance and Rusak, 1985). Completeness of lesions was determined by examining Kluver-Barrera stained sections through the geniculate area (Kluver and Barrera, 1953) as well as by assessing the density of remaining neuropeptide Y-immunoreactive fibers in the SCN and in some cases, in the geniculate area.

II.B.1.b. Specific methods for Experiment 1

This experiment was designed to assess the effect of ablation of the GST on the light pulse phase response curve. Hamsters were housed in cages placed within individual, light-tight wooden boxes (52 X 48 X 37 cm). A fluorescent light bulb (Sylvania cool white F15T12-CW) was attached to the ceiling, and a piece of white translucent Plexiglas, which acted as a diffuser,
was placed 30 cm above the cage floor. Light intensities, measured in the middle of each cage, were approximately 40 \( \mu \text{W/cm}^2 \) or 160 lux (range: 30 - 50 \( \mu \text{W/cm}^2 \)). Lighting could be controlled independently for each box. A fan was on continuously to provide ventilation and background masking noise.

Hamsters were housed in continuous darkness (DD). After a stable freerunning rhythm was established, animals were exposed to four separate light pulses, timed so as to produce intermediate or maximal phase delays or phase advances. Pulses were given at CTs 12, 14, 18 and 20, presented in a random order to each animal. If a phase shift could not be measured accurately because of instability in the rhythm, the pulse was later repeated. Once phase shifts to light pulses at each of these CTs were determined, eight hamsters were given geniculate lesions. After recovery from surgery, phase shifts to light pulses at each of the four circadian times were measured again (10 - 121 days after surgery).

Three animals received no surgery and served as controls for the effects of repeated measures; they were retested in the same manner as ablated animals. The responses of 17 additional intact hamsters to light pulses at these four circadian times were measured. These animals were not retested because of illness or
instability in their activity rhythms.

II.B.2. Results

II.B.2.a. General histological results

Neuropeptide Y-immunoreactivity in the SCN was greatly reduced in hamsters in which the IGL and the anterior vLGN were completely destroyed. A minimal amount of neuropeptide Y-immunoreactivity remained in the ventromedial SCN after complete lesions (see Harrington, Nance and Rusak, 1985). Greater amounts of neuropeptide Y-immunoreactivity in the SCN indicated incomplete lesions; this inference was confirmed in some hamsters by direct observation of surviving neuropeptide Y-containing cells in the geniculate area. Kluver-Barrera stained tissue allowed visualization of the IGL-vLGN lesions and the incidental damage to portions of the dorsal lateral geniculate nucleus, the medial geniculate nucleus, the zona incerta and the ventral thalamic nucleus (see Figure 2).

II.B.2.b. Results of Experiment 1

The pattern of response to light pulses shown by intact animals in our laboratory was consistent with a previously published 15 min light pulse phase response curve for hamsters (Daan and Pittendrigh, 1976a). The
animals which subsequently received lesions showed
pre-lesion phase shifts, that were not significantly
different from the other intact hamsters' responses
\(F(1, 59) = 3.464, p > .05\).

Six of the eight hamsters receiving lesions had
complete GST ablation. Each of the hamsters with
complete lesions given light pulses at CT18 and CT20
showed decreases in phase advance shifts after surgery
in comparison to shifts measured before surgery. At
CT18, the mean (± s.e.m.) pre-lesion shift of 273 ± 39 min
was reduced to a mean post-lesion shift of 172 ± 15 min
\((n = 5; \text{ t-test, } p < .05)\). At CT20 shifts were decreased
from a mean of 212 ± 38 min pre-lesion to a mean of 129 ±
19 min post-lesion \((n = 6; \text{ t-test, } .05 < p < .10)\).
Phase delays elicited by light at CT12 and CT14 were not
altered consistently by the lesions (see Figure 3).

Several animals did not survive long enough to
obtain retest measures at all four CTs. Because of the
small number of retest scores obtained for the three
control animals which did not receive lesions, I did not
test for statistical differences between retest values
for these animals and for those receiving lesions. The
three control animals did not show substantial changes
in the sizes of phase shifts (see Figure 3). The
largest change shown by a control hamster on retest was
a decrease of 42 min in the shift produced by a CT18
pulse; this decrease was smaller than all but one of those seen in ablated animals at that phase.

There was no change in freerunning period that could be attributed to the lesions; however, most ablated and control animals showed a gradual decrease in period over the course of the experiment (cf., Pittendrigh and Daan, 1974) Period changes were not correlated with alterations in phase shifting.

II.C. Experiment 2. Dark pulse phase response curves

II.C.1. Methods

For general methods, see section II.B.1.a.

This experiment was designed to assess the effect of GST ablation on the dark pulse phase response curve. Twenty hamsters were housed in a room with lighting provided by two fluorescent light tubes (Sylvania Daylight - F40) set vertically against a wall, 80 cm from the cages. These tubes generated light intensities of 5.5 \( \mu \text{W/cm}^2 \) or 50 lux measured in the middle of each cage (range: 5.0 - 6.0 \( \mu \text{W/cm}^2 \)). Dark pulses were administered to all hamsters by turning off the room lights for six h, or to individual hamsters by placing the hamster and its cage into a light-tight box (58 X 33 X 30 cm) for six h. Shifts were not calculated for hamsters that
showed imprecise onsets or that showed an activity rhythm split into two components approximately 12 h apart.

This experiment was run in three cohorts of hamsters. The first cohort consisted of 20 hamsters which were housed in constant darkness (DD) until stable freerunning rhythms were established (day 28 - 36); 10 hamsters then received geniculate lesions and 10 hamsters were sham-operated. On day 51, hamsters were placed in constant light (LL) as described above and subsequently exposed to 6 h dark pulses.

The second cohort consisted of 20 hamsters housed in LL. Ten of these hamsters had received geniculate lesions immediately before being placed in the room, while the remaining 10 hamsters served as intact controls. After a stable freerunning rhythm was established for most of the hamsters (day 35), a dark pulse was administered by turning the room lights off for six h. Six h dark pulses were also given on days 51, 71, and 92 to all hamsters. By day 110, seven hamsters showed a "split" pattern of activity; the room lights were then turned off and hamsters were kept in DD until day 130 in order to promote consolidation of their activity rhythms (Earnest and Turek, 1982). Animals were returned to LL on day 130 and given a final dark pulse on day 153.
The third cohort consisted of 17 hamsters housed under continuous illumination (LL). The purpose of this cohort was to further investigate the results obtained from the first two cohorts. Selected one time period at which the results from cohorts 1 and 2 suggested differences between the groups in dark pulse-induced phase shifts, \((\text{CT8} - 10)\). Seven GST-ablated and 10 sham-operated hamsters were tested for their responses to dark pulses at this time. All other procedures were identical to those for the first two cohorts.

II.C.2. Results

For general histological results, see section II.B.2.a.

In cohort 1, none of the ten hamsters which received lesions in the early part of the study had complete GST ablation. Two of the hamsters which replaced hamsters that died during the experiment did sustain complete GST ablation while five replacement hamsters had partial GST ablation. In total, five phase shifts after dark pulses were recorded from the two hamsters with complete GST ablation. Measures of 18 phase shifts after dark pulses were collected from the sham-operated hamsters in this cohort.

In cohort 2, 8 of the 10 hamsters which received lesions sustained complete GST ablation. A total of 28
Phase shifts to dark pulses were recorded from these 8 hamsters. Since the pattern of phase shifts from the cohort 2 intact hamsters did not appear to differ from that of the sham-operated hamsters in cohort 1, the data from these two groups (n = 20) were combined for phase-shift analysis, as were the data for hamsters from both cohorts with complete lesions (n = 10).

Phase shift measures were grouped into bins of two circadian hours each. Differences between the GST-ablated and control hamsters were observed only for advancing phase shifts. Control hamsters showed phase advances to pulses centered on CT5 - CT18 and phase delays to pulses centered on CT20 - CT1. The dark pulse phase response curve for the hamsters with complete lesions appeared to differ from that for the controls in that the hamsters with lesions showed smaller advances than controls to pulses centered on CT8 - 10 and CT17 - 19 (see Figure 4). A statistical analysis of these data was not possible due to the variable occurrence of repeated measures.

In cohort 3, 6 of 7 hamsters which received geniculate lesions had complete ablation of the GST. The phase shifts of these 6 hamsters to dark pulses centered on CT8 - 10 were compared to those of 10 sham-operated controls. Each animal contributed only one phase shift measure to the calculations. The mean
circadian times of the dark pulses given to hamsters of each group did not differ significantly (mean ± s.e.m.; controls: CT 9.2 ± 0.1, lesions: CT 8.9 ± 0.1; t-test, n.s.). The hamsters with complete GST ablation showed decreased phase advances to dark pulses as compared to sham-operated hamsters (mean ± s.e.m.; controls: 64.4 ± 10.9, lesions: -15.3 ± 14.9; t-test, p < 0.001), thus confirming one of the suggested differences in the data from cohorts 1 and 2.

The responses of the hamsters from cohorts 1 and 2 to the various lighting conditions were analyzed. The cohort 1 hamsters were not grouped with the cohort 2 hamsters in this analysis for several reasons. Animals from different cohorts had different photic histories. The two hamsters in cohort 1 with complete GST ablation were replacements of original hamsters and therefore had different photic histories than the control hamsters in this cohort. Finally, many of the dark pulses for cohort 1 hamsters were administered individually. Once hamsters showed disrupted activity rhythms, it was less likely that they would receive a dark pulse. Cohort 2 hamsters were treated much more uniformly since hamsters were not replaced and since most dark pulses were administered to the group.

GST-ablated hamsters differed from control hamsters in their responses to LL in two major respects; they
showed rhythms with different periods under LL, and they were less likely to show "split" rhythms. The GST-ablated hamsters in the second cohort (n = 8) differed from the control hamsters (n = 10) in their response to long-term exposure to continuous illumination. In the first 35 days of LL, before any dark pulses were administered, hamsters with complete lesions showed significantly shorter freerunning periods than did the intact controls (mean ± s.e.m., 24.07 ± .02 h (n = 8) vs. 24.26 ± .06 h (n = 10); t-test, p < .02). The two hamsters with partial lesions had a mean period of 24.16 ± 0.03 h.

Three of the cohort 2 hamsters with complete GST ablation displayed rhythms with periods of less than 24 h throughout the 110 days in LL, while no intact controls showed similarly sustained short-period rhythms. Figure 5 shows a record typical of the three hamsters with short periods. After 110 days of LL, this hamster's activity rhythm had a period of 23.77 h. One ablated hamster (see Figure 6) developed a very long period (approximately 26 h) after 60 days in LL, shortly after exposure to a dark pulse.

The second major effect of GST ablation on rhythms in LL was a decreased likelihood of "split" rhythms. After 110 days in LL, 7 of 10 intact hamsters, but none of the 8 ablated hamsters in cohort 2, showed typical
"splitting" of activity rhythms into two components approximately 12 h apart (Pittendrigh and Daan, 1975b; see Figure 7). This difference was statistically significant (Fisher's exact test, p < .01). The split pattern was apparent in control hamsters after an average of 70 ± 4 (± s.e.m.) days in LL. One GST-ablated hamster showed dissociated activity components that might have reflected incipient splitting (see Figure 8, days 95 - 110). One of the two hamsters with partial lesions in this cohort showed a split rhythm after approximately 60 days in LL. In cohort 1, the two hamsters with complete GST ablation did not show split rhythms; however, these hamsters were not exposed to LL for as long as 110 days. Of the hamsters in LL for 110 days, 6 of 10 sham-operated and 6 out of 9 partial-lesion hamsters had split rhythms. The split patterns were apparent after an average of 77 (± 12) days for the sham-operated hamsters and 73 (± 5) days for the partial-lesion hamsters (means are ± s.e.m.).

When hamsters in cohort 2 were exposed to continuous darkness (DD), all previously split rhythms fused again. Three ablated and one intact hamster showed activity rhythms with two components running with different periods under DD (see Figure 9). There was no difference between ablated and control hamsters in the period of the activity rhythms during exposure to DD.
(t-test, p = 0.52). Three hamsters with lesions had activity rhythms with periods that were longer in DD (mean period = 24.01 ± 0.05 h) than in the preceding LL (mean period = 23.76 ± 0.05 h; see Figure 5); no control hamsters showed this pattern.

Rhythms during the second exposure to LL (days 130 - 200) showed much more disruption. During this time, intact hamsters showed typical splitting (5 hamsters), disrupted activity rhythms (3 hamsters) or arrhythmicity (2 hamsters). One of the two hamsters with partial lesions split during the second exposure to LL. None of the 7 hamsters with complete lesions exposed to LL a second time showed typical splitting or arrhythmicity during this period, but six hamsters showed some disruption of activity rhythms (see Figures 5, 6, 8 and 9). Sometimes these activity rhythms appeared to have two components, but these patterns differed from typical splitting in that the patterns were never stable and the components were not coupled approximately 12 h apart as is characteristic of split rhythms. Although periods of the rhythms were rather variable during this interval, five of these six hamsters with complete lesions had activity rhythms with relatively long periods (ranging from 24.75 to 26.29 h) during at least some portion of the second exposure to LL (see Figures 5, 6 and 8).
II.D. Discussion

Hamsters with GST lesions showed responses to light and dark pulses that differed from those of control animals. Ablated animals displayed decreased phase advances to light pulses at CT18 and CT20, although no differences were observed in phase delays to pulses at CT12 and CT14. Responses to dark pulses may have been affected by GST lesions in a similar manner; phase advances to pulses centered on CT8–10 were decreased and advances to CT17–19 pulses may have been decreased also, while the small phase delays elicited by pulses centered on CT20–CT1 did not appear to differ between groups. Thus, it is possible that GST ablation selectively attenuates phase advances without affecting phase delays, regardless of the type of stimulus used to induce these phase shifts.

Electrical stimulation of the vLGN at various CTs causes a pattern of phase shifts of hamster activity rhythms that is similar to that caused by dark pulses (Meijer, Rusak, and Harrington, 1984). It has proven difficult, however, to obtain large delay shifts by such stimulation. Neuropeptide Y injections, which produce substantial phase advances, may also produce only relatively small phase delays (Albers and Ferris, 1984).
These observations support the idea that GST neurons are involved principally in modulating phase-advancing rather than phase-delaying shifts. A recent report by Ralph and Menaker (1985) demonstrated that in hamsters pretreatment with bicuculline selectively reduced the amplitude of delay shifts caused by light pulses without affecting advance shifts. These results and my data imply that the mechanisms mediating delay and advance shifts in the mammalian circadian system must diverge at some stage.

Three considerations must qualify the hypothesis that GST ablation selectively affects phase advances. First, a moderately bright light was used for the light pulses. In an experiment comparing vLGN-ablated and control hamsters, Pickard and Ralph (1984) used lower light intensities (approximately 0.128 μW/cm²; pers. comm.) than the light intensities used in this study (40 μW/cm²) and found that the ablated animals showed increased delay shifts to light pulses at CT13.5, as well as decreased advance shifts to light pulses at CT18. The higher intensity light pulses in the present study may have come close to saturating the circadian photic system. Increases in phase shifts might not have been observed because of a "ceiling" effect, while decreases could be measured readily.

A second consideration is that delay shifts to dark
pulses were quite small in control animals. Differences between ablated and control animals in delay shifts might not have been apparent because delay shifts were too small relative to the variability in shifts between animals. The dark pulse phase response curve of the controls in this study differs in this respect from that of Boulos and Rusak (1982) who reported delays as large as 5 h after 6 h dark pulses.

A third consideration is that GST ablation did not appear to alter phase advances to dark pulses centered on CT11 - CT16. The groups appeared to differ only on the rising and falling slopes of the advance portion of the dark pulse phase response curve. This does not support the hypothesis of a general effect of GST ablation on advancing phase shifts, but indicates a more phase-specific effect.

Under DD, hamsters with GST ablation showed rhythms with periods similar to those of controls; however, they displayed periods shorter than controls during an initial 35-day exposure to LL. In some animals, the period expressed in DD was actually longer than the period observed in an immediately preceding condition of LL. The latter response is atypical for hamsters, which generally show an increase in period with an increase in light intensity (Aschoff, Figala and Poppel, 1973; Daan and Pittendrigh, 1976b). Rusak and Boulos (1981)
reported that hamsters with primary optic tract lesions showed activity rhythms with longer periods in DD and LL as compared to controls (see also Rusak, 1977). The differences between these studies and the present study may be attributable to differences in the lesions used or to the different photic histories of the animals.

The relation between the effects of lesions on period and the effects on phase response curves is unclear. Under one theoretical framework (Daan and Pittendrigh, 1976b), a decrease in the advance portion of the light pulse phase response curve leads to the prediction of longer periods under LL and a more dramatic lengthening of period with increases in constant light intensity. However, the ablated animals in our study show the opposite relationship—a decrease in the advance portion of the light pulse phase response curve is associated with a shorter period in LL.

The conclusion that animals with GST ablation show decreased periods in LL must be qualified, since many ablated animals also showed extremely long periods during a second exposure to LL (see Figures 5-7). One effect of GST ablation may be to decrease stability of period under LL. Another study would be needed to determine to what degree the variability in period seen here was a response to LL and to what degree it was a response to repeated dark pulses, which often cause
changes in period (Boulos and Rusak, 1982).

Another effect of GST lesions was that ablated animals did not show typical splitting of the activity rhythm into two components in LL. Control animals did show such splitting, which is a common finding in hamsters exposed to LL (Earnest and Turek, 1982; Pittendrigh and Daan, 1976b). It is possible, but doubtful, that splitting would have been observed in some ablated animals if the second exposure to LL had been prolonged. During this time most ablated animals showed transient dissociation of activity rhythms into two or more components as well as periods longer than those observed during the first exposure to LL. Rhythms with two components, however, did not appear stable in these animals; the second component usually had disappeared by the end of the exposure to LL. When exposed to DD, several ablated hamsters displayed activity rhythms with two components with transiently different periods. This behavior is similar to that shown by intact mice (Peromyscus leucopus) exposed to DD after compression of their activity phase under LL (Pittendrigh and Daan, 1976b).

The phenomenon of splitting challenges any single-oscillator model of the circadian pacemaker. Therefore, most models which account for splitting involve two or more oscillators which are normally
coupled in a stable phase relation, but which may also show stable coupling in antiphase (Enright, 1980; Pittendrigh and Daan, 1976b). The occurrence of rhythm disruptions in DD and in the second exposure to LL in GST-ablated hamsters indicates that the absence of splitting during the initial exposure to LL is not due to a change in pacemaker structure that prevents the expression of multiple rhythmic components. Since the patterns of rhythms with several components never appeared stable in the hamsters with GST ablation, it is possible that the coupling of these components is affected by loss of the GST.

The behavioral effects of GST ablation may indicate that GST input to the SCN plays a direct role in inducing phase advancing shifts and in responses to LL. It is also possible, however, that GST input plays only an indirect role. Thus, some other input may directly induce advancing phase shifts and responses to LL, and GST input may be necessary only for SCN neurons to adequately respond to this other input. The present data do not allow these two possibilities to be distinguished.

Effects of GST ablation are presumably attributable in part to the loss of the neuropeptide Y-containing afferents to the SCN. The effects of GST ablation observed here are consistent with the idea that
neuropeptide Y is selectively involved in phase advance shifts. Recent observations, however, indicate that there are neuropeptide Y afferents to the SCN other than those originating in the IGL (Allen et al., 1984; Harrington, Nance and Rusak, 1985). Elimination of these afferents might amplify or alter the effects of IGL ablation. Such results might modify our interpretation of the role of neuropeptide Y in the SCN.

The effects of GST ablation may also be attributed in part to the damage inflicted on retinal afferents to this area. Some of these afferents are collaterals of retinal afferents to the SCN (Pickard, 1985) and their damage in the geniculate area may cause any of several structural changes in the direct retinal projection to the SCN (see Rusak and Boulos, 1981); these changes might include complete degeneration of the retinal ganglion cells whose bifurcating axons form at least part of the projection from the retina to the IGL and the SCN. Alterations of circadian rhythms might, therefore, result from a change in the photic information reaching the SCN via the direct retinohypothalamic tract rather than to a loss of GST afferents.

In summary, this study has demonstrated that ablation of the GST in hamsters produces several changes in photic responsiveness of circadian rhythms. Advance
shifts to both light and dark pulses at some phases are decreased following such lesions. Similar effects are not seen for delay shifts, implying a differential role for the GST in mediating different kinds of phase shifts. Ablated animals differ from control animals in the periods of activity rhythms during exposure to LL, and ablated animals are much less likely than controls to show "split" activity rhythms in LL.
Figure 2. Representative coronal brain sections from animals with the largest (A) and smallest (B) lesions which completely ablated the intergeniculate leaflet (IGL) and the ventral lateral geniculate nucleus (VLGN). Complete ablation of tissue is shown as dark shading while heavily gliosed areas are shown as lighter hatching. (Abbreviations: dorsal lateral geniculate nucleus (DLGN), hippocampus (HIPP), medial geniculate nucleus (MGN), optic tract (OT). Magnification = 12 X.)
Figure 3. Mean (+/- s.e.m.) difference between pre- and post-lesion phase shifts to 15 min light pulses given at various circadian times (CT). Differences between first and second measures of phase shifts for animals which did not receive lesions are also shown (x's).
Figure 4. Mean (+/- s.e.m.) phase shift to 6 h dark pulses for control hamsters (solid line) and for hamsters with complete GST ablation (dashed line). Shifts were grouped into two CT h bins. Phase advances are shown as positive values, while phase delays are indicated by negative values.
Figure 5. Double-plotted running wheel activity of a hamster with complete GST ablation in Experiment 2. From day 0 to day 110, the animal was housed under constant illumination (LL). It was then housed in constant darkness (DD) for twenty days, following which it was returned to LL until the end of the experiment. Six h dark pulses were given on days 35, 51, 71, 92, and 153; these are indicated by black rectangles on the right side of the figure.
Figure 6. Double-plotted running wheel activity of a hamster with complete GST ablation. See Figure 5 for further explanation.
Figure 7. Double-plotted running wheel activity of an intact hamster. See Figure 5 for further explanation.
Figure 8. Double-plotted running wheel activity of a hamster with a complete intergeniculate leaflet lesion. See Figure 5 for further explanation.
Figure 9. Double-plotted running wheel activity of a hamster with complete GST ablation. See Figure 5 for further explanation.
CHAPTER III. Characterization of neuropeptide Y immunoreactivity of and retinal input to GST neurons

III.A. Introduction and literature review

This chapter will describe anatomical experiments characterizing the relationship between neuropeptide Y and the neurons of the GST. Double-labeling and fusion studies are used to establish that neuropeptide Y is found in many, if not all, of the GST neurons in the hamster. The location of GST neurons in the intergeniculate leaflet (IGL) and vLGN and the pattern of retinal input to these neurons is described.

In the first section of the introduction, I will describe the cytoarchitecture of the IGL and the vLGN. The second section will describe the known afferent and efferent connections of this area. Retinal innervation of the IGL and vLGN will be treated separately, in a third section. The fourth section will review anatomical research on the GST and the fifth section will provide information about neuropeptide Y and other related peptides. The final section will introduce and describe the rationale of the experiments to follow.

III.A.1. Cytoarchitecture of the LGN

The hamster lateral geniculate nucleus can be
separated into three divisions: the dorsal lateral geniculate, the intergeniculate leaflet and the ventral lateral geniculate (see Figure 10). The dorsal lateral geniculate nucleus (dLGN), part of the dorsal thalamus, receives direct retinal input and projects mainly to the visual cortex. The intergeniculate leaflet (IGL) is a thin lamina of neurons located between the dorsal and the ventral lateral geniculate nuclei, extending caudally and medially into the zona incerta. This lamina contains many of the neurons of the GST. The vLGN, derived from the ventral thalamus (Altman and Bayer, 1979), may be further subdivided into an internal division and an external division, with the external division having both an internal part and an external part (vLGNi, vLNGei and vLNGee; see Figure 10). Some neurons projecting to the SCN are found in the rostral vLNGei. The labeling of subdivisions of the LGN as shown in Figure 10 will be that used in the remainder of this thesis. In this section I will review the background of literature on cytoarchitectural divisions of this area.

An early scheme of subdivision of the vLGN (Niimi, Kanaseki and Takimoto, 1963) proposed two parts: an external division and an internal division, with the internal division containing smaller cells which were less deeply Nissl stained than those in the external
division. These two subdivisions are separated by a group of fine fibers and a neuron-sparse zone. This general scheme was confirmed in the rat by Swanson et al. (1974).

In some species, the external division may be further divided on the basis of cell type into the vLGNee and vLGNei (hamster: Frost et al., 1979; rabbit: Holcombe and Guilléry, 1984). The vLGNei is distinguished by its larger and more densely packed neurons.

The IGL was first described by Hickey and Spear (1976) in the course of a study of the retinal projections to the geniculate in the rat. The IGL encompasses what previous studies had referred to as the internal dorsal division of the vLGN or as the dorsal vLGN. Takahashi et al. (1977), working from material with labeled retinal afferents, described the IGL in rabbits. Feldman and Kruger (1980) described the IGL in rats from material with the medial lemniscus labeled with autoradiography; they called it the "intermediate nucleus of the geniculate". The IGL is also clearly defined in some immunocytochemical material as in described in section III.A.2.

The cytoarchitecture of the vLGN in the cat appears to differ from that of rodents (Jordan and Hollander, 1972). The vLGN of the cat is shaped like a question
mark, with a broad main part dorsally and a slender stalk which is twisted around the rostrolateral aspect of the optic tract. Five subdivisions of the nucleus were identified by Jordan and Hollander; these were labeled with the letters a - e (e.g., vLGNa, etc.).

Jordan and Hollander's cytoarchitectural scheme for the cat vLGN does not correspond exactly with the more general plan suggested by Niimi et al. (1963) of an internal and external division. The external part of the external division in Niimi's scheme would probably correspond to the vLGNe, and the internal division to vLGNb in Jordan and Hollander's scheme. Since the two schemes are not entirely compatible much of the anatomical work on the vLGN of the cat cannot be related directly to the vLGN of rodents.

Several studies have attempted to classify the neuron types in the vLGN and IGL of the rat on the basis of Nissl and Golgi stains (Brauer et al., 1984; Mounty et al., 1977; Stelzner et al., 1976). The classification scheme of Brauer et al. (1984) appears to be the most consistent and comprehensive scheme. Five classes of neurons in the vLGN and IGL were described (see Table 1).

Descriptions of branching patterns of dendrites of Golgi-stained neurons in the vLGN and IGL of the rat (Mounty et al., 1977) raise the possibility of
communication across the subdivisions of the vLGN. In
the vLGN some neurons were observed to have long
dendrites extending dorso-ventrally. Many extended into
the IGL, and some extended as far as the dorsal lateral
geniculate. In the ventral parts of the vLGN some very
large neurons had dendrites oriented perpendicular to
the optic tract, sometimes spanning the entire
medio-lateral axis of the vLGN.

Electron microscopic studies of the vLGN indicate an
organization somewhat similar to that of the dorsal
lateral geniculate. Four types of synapses have been
identified in the vLGN (Stelzner et al., 1973, 1976).
"Synaptic islands" consisting of 3 or more synapses are
observed throughout the vLGN (Stelzner et al., 1976).
These differ from the "synaptic glomeruli" found in
other thalamic nuclei (Jones, 1983) in that they are not
encapsulated by glial processes and the component parts
are more variable. In the vLGN, synaptic islands do not
appear to include direct cortical input. It appears
that the neurons which are the main recipients of
retinal input may also receive inhibitory input (as
indicated by symmetrical synaptic junctions) close to or
on the soma.

III.A.2: Afferent and efferent connections of the vLGN
and IGL

A major source of afferent input to the vLGN and IGL is from the retina. This projection will be discussed in section III.A.3. Information about afferents to the intergeniculate leaflet other than those from the retina would aid in developing hypotheses about its function. Unfortunately, in many studies, the IGL was not distinguished from the vLGN. Thus, it is not clear if these studies provide information relevant to the GST neurons of interest, which are mainly located in the IGL. I have summarized in Tables 2 and 3 the major afferent and efferent projections of the vLGN-IGL area. These projections are discussed in detail in Appendix A. In the remainder of this section, I will summarize only what is known about the afferent and efferent connections of the IGL specifically. Most of the studies referred to below are discussed in detail in Appendix A.

It appears that IGL class 3 neurons (see Table 1) project to the contralateral IGL (Brauer et al., 1984; Cosenza and Moore, 1984; Graybiel, 1974; Mackay-Sim et al., 1983; Pasquier and Villar, 1982a; Pickard, 1982, 1983; Swanson et al., 1974; but see Ribak and Peters, 1975), while class 1 IGL neurons project to the pretectum (Schober, 1983) and class 4 neurons project to the pons (Brauer et al., 1984, pers. comm.). As will be
described later in this chapter, the neurons in the IGL which are neuropeptide Y-immunoreactive and project to the SCN appear to be mainly class 4 neurons, although some may be class 3 neurons. From the morphological similarities, one might hypothesize that IGL neurons that project to the SCN also project to the pars and perhaps to the contralateral IGL as well (but see Pickard, 1985).

Input to the rat's IGL comes from the locus coeruleus (Kromer and Moore, 1980); medial lemniscus (Feldman and Kruger, 1980), zona incerta (Watanabe and Kawana, 1982) and the midbrain central gray (Eberhart et al., 1985). This nucleus is further characterized in rats by cells and fibers staining for acetylcholinesterase (Brauer et al., 1984) and enkephalin (Mantyh and Kemp, 1983), fibers staining for serotonin (Cropper et al., 1984; Mantyh and Kemp, 1983), noradrenaline (Kromer and Moore, 1980) and glutamic acid decarboxylase (Ohara et al., 1983), and substance P fibers (Mantyh and Kemp, 1983) and receptors (Mantyh et al., 1984). Little is known about the source of these various neurotransmitters and peptides, except that it is likely that the serotonin comes from neurons in the raphe nuclei (Pasquier and Villar, 1982b), the noradrenaline from the locus coeruleus (Kromer and Moore, 1980) and the acetylcholinesterase from the
midbrain reticular formation (Hoover and Jacobowitz, 1979).

III.A.3. Retinal innervation of the vLGN

The vLGN receives a direct retinal projection in all species studied to date; however, the details of the pattern of termination of the retinal afferents within the vLGN vary between species (see Table 4). One of the earliest relevant reports of a study, making use of autoradiographic tracing techniques (Swanson, Cowan and Jones, 1974) claimed that in rats a heavy retinal projection was observed over the contralateral vLGN, while light labeling was seen over the medial edge of the caudal vLGN on the contralateral side.

A more complete study by Hickey and Spear (1976) compared the retinal afferents to the vLGN in albino and hooded rats, also using autoradiography. The patterns of termination they described are much more extensive than those noted by Swanson et al. (1974). As in the preceding report, a heavy contralateral projection to the vLGN was observed. Patches of retinal terminal fields were observed in the ipsilateral vLGN. It was possible that these patches corresponded with regions of reduced retinal projections on the contralateral side. Some fibers appeared to terminate in the vLGN at caudal
levels. There were no substantial differences between the two strains of rats in their patterns of retinal terminations in the vLGN.

Hickey and Spear also identified a bilateral retinal projection not described by Swanson et al. (1974), terminating in the IGL. This retinal projection was confined rostrally to the region between the dLGN and the vLGN, but at more caudal levels, it appeared to extend ventrally, merging with the zona incerta just lateral to the medial lemniscus. The contralateral projection to the IGL was somewhat heavier than the ipsilateral. Although they reported great variability between animals in the size and location of this projection, it appeared that the contralateral and ipsilateral projections overlap, especially at more caudal levels.

The retinal afferents to the vLGN and IGL in the rabbit were described by Takahashi et al. (1977). The IGL receives an overlapping bilateral retinal projection. In the vLGN, the contralateral eye projects mainly to the external division, but also sends a light projection to the internal division, mainly along its dorsal and lateral borders. The ipsilateral eye projects to an area along the dorsal border of the external division. Most of the conclusions of Takahashi et al. (1977) were confirmed by Holcombe and Guillery.
(1984). In addition, they described a topographic organization of the retinal projection to the vLGNe. The IGL, on the other hand, showed no evidence of a topographically organized retinal input. The retinal input to the vLGNe and vLGNei were compared in Fink-Heimer stained material. It appeared that degenerating retinal fibers in the vLGNe were of finer calibre than those in the vLGNei, indicating that these two areas may receive retinal input from axons of different calibre, possibly with different physiological properties.

The retinal projection to the vLG in hamsters was shown to be topographically organized in a study of degeneration patterns after focal retinal lesions (Jhaveri and Schneider, 1974). The representation of the temporal retina is in the dorsal vLG, while nasal retina is represented ventrally. In a study using both radioactively labeled amino acid transport and anterograde degeneration techniques, Frost, So and Schneider (1979) examined the location of retinal terminals in the hamster vLG. As in other species, the contralateral vLGNe was observed to receive a dense retinal projection. Small patches of retinal terminations in the ipsilateral vLGNe, similar to those seen in rats by Hickey and Spear (1976) were also noted. In hamsters, however, these patches were specifically
Confined to the medial extremity of the internal lamina of the vLGNe. In addition, a sparse bilateral projection to the IGL was seen, especially at more caudal levels. Although the projections from the two eyes to the IGL seemed to be completely overlapping, the degree of overlap of the retinal projections to the vLGNe could not be determined.

The retinal projection to the vLGNe of the 13-lined ground squirrel was studied by Kicliter and Bruce (1983) using autoradiographic methods. Retinal projections were mainly confined to the vLGNe and the IGL. Based on the laterality of the projections, five regions could be distinguished. Three regions receiving projections from the contralateral eye were interleaved with two regions receiving ipsilateral projections. The IGL received both contralateral and ipsilateral projections, the separation of which was most marked rostrally. The vLGNe also received a bilateral retinal projection. It may be concluded from their figure that the vLGNe receives a sparse bilateral retinal projection, with that from the contralateral eye being heavier than the ipsilateral. Kicliter and Bruce's results are interesting in that the ipsi- and contralateral retinal projections to the IGL and the vLGNe were found to be mainly non-overlapping. Such a laminated pattern of retinal afferents to the vLGNe had not been reported previously. However, this
pattern should be confirmed with more appropriate material, such as that from an animal with different anterograde tracers placed in each eye.

To summarize, it appears that in many species both the vLGN and the IGL receive retinal projections from both eyes, although those from the contralateral eye are usually heavier. Most authors do not distinguish between the external and internal divisions of the vLGN; therefore, the differences noted in this projection in the hamster by Frost et al. (1979) cannot be safely generalized. It is difficult to generalize about the retinal projection to the vLGNi, except that, if it exists, it is a very sparse projection (see Table 4).

III.A.4. The geniculo-suprachiasmatic tract

The existence of the geniculo-suprachiasmatic tract was first demonstrated by Swanson, Cowan and Jones (1974). They described the pattern of labeling seen after injections of tritiated proline or leucine in the vLGN of rats and cats. One prominent pathway was described in the rat as coursing ventrally toward the ipsilateral lateral terminal nucleus which was heavily labeled. It then proceeded through the zona incerta (where some fibers may have terminated) and, passing
ventral to the medial lemniscus, came to terminate bilaterally in the ventral half of both SCN. The ipsilateral suprachiasmatic nucleus contained approximately twice as many silver grains as the contralateral nucleus. In the cat, this projection was similar except that the fibers leaving the vLGN passed anteromedially, going through the ipsilateral reticular nucleus.

Ribak and Peters (1975), who also used autoradiography to determine the efferent projections of the vLGN in rats, reported a different route for vLGN fibers coursing to the SCN. In their study, labeled fibers were observed to descend in the optic tract, cross in the ventral supraoptic (Meynert's) commissure and terminate bilaterally in the SCN.

In an effort to resolve this controversy, Legg (1979) injected labeled amino acids into the vLGN of rats and allowed a long (12 day) survival time. This allowed better visualization of fiber pathways. Only one rat in this study had a well placed injection, but in this case a labeled pathway was observed following the optic tract as described by Ribak and Peters (1975). Thus, it is most likely that the geniculo-suprachiasmatic tract descends along the optic tract and joins the fibers of Meynert's commissure. It is probable that Swanson et al. (1974) observed labeled
entire fostro-caudal

"Extent of /theIGL. The labeled™

Pickard (1982), undertook a detailed study of the cells of origin of the geniculo-suprachiasmatic tract. Horseradish peroxidase (HRP), a retrograde tracer, was injected into the SCN either unilaterally or bilaterally in hamsters. Labeled neurons were observed in the entire rostro-caudal extent of the IGL. The labeled neurons and their dendritic processes were usually oriented perpendicular to the retinal axons traversing the area. A small number of labeled neurons were also observed throughout the vLGNI. Labeled neurons were never detected in the vLGNe. The vLGNI input to the SCN was bilateral, with approximately 2 to 3 times as many labeled neurons found in the ipsilateral vLGNI after a unilateral HRP injection. Labeled neurons afferent to the SCN were also noted directly caudal to these vLGNI neurons, in the area of the zona incerta and the lateral terminal nucleus. It is likely that these neurons correspond to the caudal tip of the IGL. Since it appeared that HRP injections into any section of the SCN labeled IGL neurons, Pickard concluded that in the hamster, the IGL innervates the entire SCN, in contrast to the rat where this input is confined to the.
ventrolateral aspect of the SCN (Swanson et al., 1974; Ribak and Peters, 1975). Some HRP injections in the hypothalamus but outside the SCN labeled retinal ganglion cells, yet failed to label IGL neurons. Thus, it appears that in the hamster retinal afferents to the hypothalamus have a wider terminal field than do IGL afferents.

Immunocytochemical characterization of the geniculo-suprachiasmatic tract in rats was reported by Card and Moore (1982). The SCN of the rat was observed to contain a dense plexus of avian pancreatic polypeptide- (APP-) immunoreactive axons confined to the ventral and lateral parts of the nucleus. A moderate plexus of immunoreactive fibers encapsulated the nucleus. Because the pattern of distribution of these fibers corresponded to the pattern of the terminal fields of both the retinohypothalamic tract and the geniculo-suprachiasmatic tract, the retina and the geniculate were both examined for APP-immunoreactive cell bodies. APP-immunoreactive perikarya were not found in any portion of the retina and, furthermore, bilateral enucleation resulted in no change in the density or distribution of APP-immunoreactive SCN axons. In the vLGN, APP-immunoreactive neurons were observed and found to be confined to the IGL. The axons of these neurons appeared to extend into the zona incerta, while
labeled axons were never observed in the optic tracts. Bilateral vLGN lesions resulted in a total loss of immunoreactive axons in the SCN, while not affecting the distribution or density of immunoreactive axons within the periventricular nucleus and anterior hypothalamic nucleus adjacent to the SCN. Unilateral vLGN lesions reduced the number of immunoreactive axons in the SCN ipsilateral to the lesion by 60 - 70%, with the contralateral side showing much less of a loss.

These findings suggest that the IGL sends a chemically distinct projection to the SCN in rats. The apparent path of APP-immunoreactive projection fibers observed is similar to that described by Swanson et al. (1974) but differs from that of Ribak and Peters (1975) and Legg (1979); however, the observation of APP immunoreactive fibers in the zona incerta and not in the optic tract is not strong enough evidence to resolve this controversy. Disappearance of labeled fibers in the zona incerta after vLGN lesions would lend weight to their conclusion, yet this result was not reported. It was noted that vLGN lesions did not noticeably affect labeling of fibers in the retrochiasmatic area nor in the commissures, yet this may indicate only that the geniculo-suprachiasmatic tract axons are a minor component of fibers in this area in the rat.

Card and Moore (1984) have reported on the
distribution of APP immunoreactivity in the SCN of the hamster. They observed that APP immunoreactivity was restricted to thin varicose axons in the ventral and lateral aspects of each nucleus. No immunoreactive perikarya were present in any portion of the SCN. At rostral levels, a moderate plexus of thin fibers was observed immediately adjacent to the interface of the SCN and the optic chiasm. At intermediate levels, this plexus expands dorsally and laterally within the ventrolateral SCN to form a dense axonal plexus which partially overlaps the field of retinal afferents, which appear to extend farther dorsally than the APP fibers. This pattern continues into the caudal pole of each nucleus. At all levels, the dorsomedial SCN is entirely devoid of immunoreactive axons. Thus, although Pickard's work (1982) suggested that the vLGN innervates the entire SCN in hamsters, Card and Moore's work indicated that if the APP-immunoreactive fibers constitute all of the vLGN afferents to the SCN, then the vLGN afferents do not innervate the dorsomedial SCN.

Pickard (1985) reported a double-labeling study using hamsters which indicated that (1) some retinal ganglion cells project to both the SCN and the LGN, and (2) IGL neurons project to either the SCN or the contralateral IGL, but not both. Double-labeling was achieved by injections of different fluorescent
retrograde tracers into the SCN and into the dLGN. Some retinal ganglion cells were clearly double-labeled. However, the double-labeled cells never made up more than 10% of the retinal ganglion cells labeled by SCN injections. No double-labeled cells were observed after control injections into the dLGN or the superior colliculus or after injections which missed the SCN. These results indicate that collaterals of at least some retinal afferents to the SCN project to the LGN. This observation implies that geniculate damage may indirectly affect the retinal projection to the SCN.

III.A.5. Neuropeptide Y and related peptides

Neuropeptide Y, a 36-amino acid peptide named after its terminal tyrosine (Y = tyrosine), shares considerable (56%) sequence homology with another 36-amino acid, avian pancreatic polypeptide (APP; see Table 5). Molluscan cardioexcitatory peptide (FMRFamide) is structurally similar to neuropeptide Y and APP in the C-terminal. Antisera to FMRFamide, neuropeptide Y and APP stain the same neurons in rat brain (Moore et al., 1984), indicating that the C-terminal is at least one common antigenic site and that the presence of tyrosine (see Table 5) is not essential for antigenic recognition.
Recent work has indicated that most of the APP and FMRF amide immunoreactivity observed in the rat's nervous system may be attributable to these antibodies cross-reacting with neuropeptide Y substrate (Allen et al., 1983; DiMaggio et al., 1985; Moore et al., 1984). Cross-blocking studies with neuropeptide Y, APP and FMRF-amide antisera and antigens indicate that neuropeptide Y is probably the peptide present in the GST of the rat (Moore et al., 1984). However, a recent report (Inui et al., 1985) indicates that pancreatic polypeptide-like immunoreactivity in porcine and canine brain may be due to antisera reacting with both neuropeptide Y and smaller amounts of endogenous pancreatic polypeptide. It would therefore be useful to perform similar cross-blocking studies in hamsters to determine the endogenous peptide in the hamster GST.

Neuropeptide Y has been shown to be colocalized with other putative neurotransmitters in some central nervous system neurons. Reports of neuropeptide Y colocalized with norepinephrine are most common, but neuropeptide Y-immunoreactivity has also been reported in neurons immunoreactive for somatostatin, enkephalin and GABA (see O'Donohue et al., 1985 for review).

III.A.6. Introduction to anatomical experiments on GST neurons
The research of Card and Moore (1982) indicating that neuropeptide Y may identify the geniculo-suprachiasmatic tract in the rat will be extended here. The experiments to follow will use two methods to determine if there is a reliable correspondence between neuropeptide Y-immunoreactivity and a projection to the SCN in geniculate neurons. First, ablation of the geniculate area will be shown to deplete neuropeptide Y-immunoreactivity in the SCN. Second, geniculate neurons will be double-labeled with both neuropeptide Y-immunoreactivity and with a retrograde tracer transported from terminals in the SCN. A practical benefit of this research is that it allows a method of assessing lesions used in other studies (e.g., in chapter II) for the extent of damage to the GST.

The second aim of the experiments described below is to give detailed information on the location of neuropeptide Y-immunoreactive neurons in the geniculate and on their pattern of retinal afference. From the results of the first experiments we can assume that at least some of these neuropeptide Y-immunoreactive neurons constitute the GST. The information on location and retinal afferents to the GST neurons is especially essential for interpretation of the electrophysiological experiments discussed in chapter IV.
III.B. Experiment 3: Neuropeptide Y immunoreactivity in GST neurons

III.B.1. The effect of geniculate ablation on neuropeptide Y immunoreactivity in the SCN

III.B.1.a. General methods

The distributions of neuropeptide Y immunoreactivity was examined in the suprachiasmatic nucleus and the geniculate area of male golden hamsters (115 - 145 g body weight). Following a lethal dose of sodium pentobarbital, animals were perfused intracardially with a vasodilating rinse of 1% buffered sodium nitrite, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2 - 7.4). The brains were postfixed in cold paraformaldehyde solution for 4 hours and stored overnight in cold 30% sucrose phosphate-buffered solution at 4°C. Serial coronal sections were cut at 40 μm with a freezing microtome. Sections were collected in 0.01 M phosphate buffered saline (PBS), rinsed several times, and then agitated overnight at room temperature with the antibody diluted in PBS with 1% triton X100 and 1% normal goat serum. The antibody used was rabbit antiserum to natural porcine neuropeptide Y (diluted 1:3750, courtesy of J.M. Polak). Localization
of the antibody was visualized using the Vectastain kit (Vector Labs), and avidin-biotin procedure (Hsu et al., 1981). Diaminobenzidine was used as a chromagen and glucose oxidase was used as the activator. The tissue was rinsed several times with PBS between each solution change. Sections were mounted on chrome-alum-dipped slides, allowed to air dry, and coverslipped with DPX or Permount after dehydration. Alternate sections of some brains were stained using the method of Kluver and Barrera (1953) for staining cells and fibers.

The distribution of neuropeptide Y in the suprachiasmatic nucleus was determined by examining the tissue of five animals. Using tissue from two animals, specificity of the antisera for neuropeptide Y was tested by incubation of the tissue in antisera preabsorbed with the antigen (Peninsula Laboratories; 100 μg of antigen per 1 ml of diluted antibody) for 60 minutes prior to reacting as described above. It was necessary to add 50% acetic acid to the neuropeptide Y antigen (50 μl per 100 μg neuropeptide Y) before dilution in phosphate buffer to insure that the neuropeptide Y was completely dissolved.

In order to facilitate the visualization of immunoreactive cell bodies, five hamsters received an injection of colchicine (60 – 200 μg colchicine in 20 μl distilled water; Sigma) into the lateral ventricle under
sodium pentobarbital anesthesia (80 mg/kg) 24 hours prior to sacrifice. "The coordinates used were 0.5 mm caudal and 1.6 mm lateral to bregma, 3.7 mm below dura."

III.B.1.b. Radiofrequency lesions of the geniculate

Twenty-eight hamsters were given bilateral lesions of the VLGN and intergeniculate leaflet. In addition, 2 animals were given unilateral lesions in order to assess how much of the geniculo-suprachiasmatic tract is crossed. Hamsters were anesthetized with sodium pentobarbital (80 mg/kg) and positioned in a Kopf stereotaxic instrument with the incisor bar 2.0 mm below the interaural line. Radiofrequency lesions were placed at three rostrocaudal points (1.6, 1.1, and 0.6 mm caudal to bregma; 3.0, 3.15, and 3.2 mm lateral to the midline; and 5.0, 4.65, and 4.38 mm ventral from dura) using a Grass LM4 lesion maker. Current equivalent to 12 to 15 mA DC was passed for 15 seconds through an electrode constructed from a stainless steel insect pin insulated except for 5 mm at the tip. Animals with bilateral geniculate lesions were sacrificed from 8 to 300 days after surgery. The two animals which received unilateral geniculate lesions were sacrificed 13 and 30 days after surgery. Alternate sections through the suprachiasmatic nucleus were processed for neuropeptide Y-immunoreactivity as described above or stained with
cresyl violet and luxol fast blue. Lesions were assessed by comparing the distribution of neuropeptide Y immunoreactivity in every third section through the geniculate area with the alternate counterstained sections.

III.B.1.c. Results

Neuropeptide Y-immunoreactive fibers forming a dense plexus in the suprachiasmatic nucleus were visualized with or without colchicine pretreatment. At both rostral and caudal levels of the suprachiasmatic nucleus, these fibers were densest ventrally, while at the middle of the rostro-caudal extent, fibers were most concentrated ventromedially. Fiber density appeared highest at these middle levels of the suprachiasmatic nucleus. Neuropeptide Y-immunoreactive fibers were observed throughout the rest of the suprachiasmatic nucleus; however, the dorsomedial suprachiasmatic nucleus showed only very sparse immunoreactive fibers. At all levels, the suprachiasmatic nucleus was bounded laterally by a "capsule" containing very few immunoreactive fibers. The capsule was most visible at middle levels of the suprachiasmatic nucleus (see Figure 11A). No immunoreactive fibers were observed in the suprachiasmatic nucleus after preabsorption of the antiserum with neuropeptide Y.
Sixteen hamsters had complete bilateral lesions of the intergeniculate leaflet and the ventral lateral geniculate nucleus as assessed by Kluver-Barrera stained tissue. No neuropeptide Y-immunoreactive cells were observed in the geniculate area of these animals. There was a marked reduction in neuropeptide Y immunoreactivity in the suprachiasmatic nucleus, although neuropeptide Y-immunoreactive fibers were still observed in the ventromedial SCN at the middle of its rostro-caudal extent (see Figure 11B). A representative lesion is shown in Figure 12. Unilateral geniculate lesions resulted in a greater reduction in the density of neuropeptide Y-immunoreactive fibers in the suprachiasmatic nucleus on the side ipsilateral to the lesion relative to that in the suprachiasmatic nucleus on the side contralateral to the lesion (see Figure 11C). The neuropeptide Y-immunoreactivity in the suprachiasmatic nucleus ipsilateral to the unilateral geniculate lesion appeared to be greater in density and extent than that remaining after bilateral geniculate lesions.

III.B.2. Double-labeling of neuropeptide Y-immunoreactive neurons which project from the geniculate to the SCN
This part of experiment 3 was designed to provide direct evidence that neurons in the geniculate area containing neuropeptide Y project to the SCN. First, it was determined that neuropeptide Y-immunoreactive neurons in the geniculate area are morphologically indistinguishable from the geniculate neurons that project to the SCN. Second, geniculate neurons were double-labeled with neuropeptide Y-immunoreactivity and with a retrograde tracer transported from an injection site in the SCN.

III.B.2.a. Methods

Sixteen hamsters received injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) aimed at the SCN. Animals were anesthetized with sodium pentobarbital (80 mg/kg) and positioned in a Kopf stereotaxic instrument with the incisor bar set 2.0 mm below the interaural line. Glass micropipettes (outside tip diameter broken to 24-80 μm) filled with WGA-HRP (15% or 1% solution in Tris buffer, pH 7.4; Sigma) were positioned 0.7 mm anterior and 0.15 mm lateral to bregma, and lowered 7.5 mm below the dura. WGA-HRP was pressure-injected by applying 0.8 - 8.0 psi for 10 - 30 seconds using a pneumatic pump (PPM-2; Medical Systems Corp.). The micropipette was removed 2 - 5 min after the injection. Animals were sacrificed with a lethal
dose of sodium pentobarbital 24 - 48 h later, and were then perfused intracardially with a vasodilating rinse of cold 1% buffered sodium nitrite. This was followed by approximately 500 ml of cold 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2 - 7.4). Brains were postfixed in a cold-1.5% glutaraldehyde - 20% buffered sucrose solution for 4 h and stored overnight in 30% buffered sucrose solution at 4°C. Coronal sections cut at 40 μm on a freezing microtome were processed for WGA-HRP using the tetramethyl benzidine (TMB) reaction (Mesulam, 1978).

Neurons immunoreactive to neuropeptide Y were examined in the geniculate area of one male golden hamster. In order to facilitate the visualization of immunoreactive perikarya, this hamster received an injection of colchicine into the lateral ventricle (see section III.B.1.a.) 24 h prior to sacrifice. After the hamster was deeply anesthetized and perfused with 4% paraformaldehyde, the brain was postfixed for 4 h and placed in a buffered 30% sucrose solution overnight. Serial coronal sections were cut at 40 μm on a freezing microtome and processed for neuropeptide Y-immunoreactivity using previously described techniques (see section III.B.1.a.). Alternate sections were treated with the method of Kluver and Barrera (1953) to stain cells and fibers.
Four additional hamsters received injections of WGA-HRP aimed at one SCN and 24 h later received intraventricular injections of colchicine (see section III.B.1.a.) either on the side ipsilateral to the WGA-HRP injections (2 cases) or bilaterally (2 cases). They were deeply anesthetized and perfused 24 h later with 3% paraformaldehyde - 0.5% glutaraldehyde as the primary fixative and the postfixing solution. Tissue was processed for WGA-HRP as described above and the TMBl reaction product was stabilized (Rye, Saper and Wainer, 1984). Sections through the geniculate area were then processed for neuropeptide Y-immunoreactivity using 3-amino-9-ethylcarbozole as the chromagen (see section III.B.1.a.). Sections were mounted on chrome-alum dipped slides, allowed to air dry, and coverslipped in glycerin diluted 4:1 with 0.1 M phosphate buffer.

Two hamsters received injections of true blue (2%, D.O. Dann) aimed at the SCN. Surgery was performed as described above and 2 μl was bilaterally pressure-injected using a 0.5 μl Hamilton syringe (Hamilton Co., Reno, Nevada). After 72 h, colchicine (150 μg in 15 μl distilled water) was injected bilaterally into the lateral ventricles (see section III.B.1.a.). The hamsters were deeply anesthetized and perfused with 4% paraformaldehyde 24 h later. Brains were postfixed for 24 h and coronal sections were cut at 50 μm using a
vibratome. Tissue was incubated in neuropeptide Y antiserum (diluted 1:500 in PBS containing 2% triton X 100 and 2% normal goat serum) for 24 h.

The immunoreactive neurons were visualized using immunofluorescent techniques. Following a 1 h incubation in biotin labeled goat anti-rabbit serum (Vector Labs), the sections were incubated for 1 h in fluorescein labeled avidin (20 μg/ml; Vector Labs). Sections were subsequently coverslipped with glycerin/phosphate buffer and visualized by epi- and transmitted fluorescence with a Leitz microscope equipped with a 200 W mercury lamp. True blue labeled neurons were visualized with a UG1 excitor and 430 barrier filter or with filter block A. The neuropeptide Y-immunoreactive neurons were visualized with an I2 filter cube.

III. B. 2.b. Results

Two of the sixteen hamsters which received WGA-HRP injections had injection sites which involved the SCN unilaterally and spread minimally to other hypothalamic structures (see Figure 13). In these hamsters, labeled neurons were observed in the intergeniculate leaflet, confirming the report of Pickard (1982). In addition, labeled neurons were observed just rostral to the intergeniculate leaflet, in the internal lamina of the
external division of the anterior ventral lateral geniculate nucleus (anterior vLGN). Two hamsters had small injection sites involving only the dorsomedial subdivision of the SCN and no labeled cells were observed in the geniculate area after these injections.

Four hamsters had larger WGA-HRP injection sites, involving the SCN, the optic chiasm, nearby hypothalamic nuclei and some spread into the ventricles. Labeling of neurons in the geniculate area was similar to that observed in the brains of hamsters with more restricted SCN injections, except that some labeled cells were also observed in the internal division of the vLGN. I concluded that these vLGN neurons project to targets other than the SCN. Eight hamsters had injection sites which did not involve the SCN and there were no labeled neurons in the geniculate areas of these brains.

The brain of one hamster with a WGA-HRP injection localized to one SCN was chosen for detailed analysis. Sections through this brain were counterstained (Kluver and Barrera, 1953) after stabilization of the TMB reaction product to allow better visualization of the labeled cells. All labeled cell bodies in the geniculate area (n=105) were drawn with a camera lucida and an oil immersion objective (final magnification = 1800 X). All neuropeptide Y-immunoreactive cell bodies (n=69) in the geniculate area of one colchicine-treated
animal were drawn under the same magnification. The traced cells were then measured using an Apple IIe graphics tablet and a stereometric measurement program (Scientific MicroPrograms).

Figure 14 illustrates the distribution of cross sectional area measurements for the cell bodies labeled with either WGA-HRP or neuropeptide Y-immunoreactivity. These distributions did not differ significantly (medians of 93 µm² and 96 µm² respectively; Mann-Whitney U test, n.s.). The medians of the maximum soma diameter measures were 18 µm for the WGA-HRP labeled neurons and 17 µm for the neuropeptide Y-immunoreactive neurons (Mann-Whitney U test, n.s.). The WGA-HRP labeled neurons did not differ from the neuropeptide Y-immunoreactive cells in the number of visible dendrites (see Table 6; Chi square test, n.s.). Most labeled neurons appeared similar to either the elongated, bipolar class 4 neurons or the oval, medium-sized class 3 neurons described by Brauer et al. (1984; see Table 1). No differences were observed in the location of neurons labeled with the two techniques. Labeled neurons in the rostral vLGNei did not differ from those in the IGL except in orientation. The long axes of neurons in the rostral vLGNei were rotated approximately 90 degrees relative to those in the IGL. No difference in orientation was observed between
neurons labeled with neuropeptide Y-immunoreactivity and those labeled with WGA-HRP (F-test, n.s.). Thus there were no systematic differences in the morphology or location of the neurons labeled by neuropeptide Y-immunoreactivity or by WGA-HRP injections into the SCN.

Two of the four hamsters processed for both neuropeptide Y-immunoreactivity and WGA-HRP labeling had injection sites which included the SCN. Many double-labeled neurons were observed in the geniculate. All WGA-HRP-labeled neurons in the geniculate area also showed neuropeptide Y immunoreactivity. A few immunoreactive neurons, however, did not show WGA-HRP labeling. Double-labeled neurons were found both in the intergeniculate leaflet and in the anterior vLGNei. Since the tissue could not be dehydrated due to the use of the water soluble chromagen 3-amino-9-ethylcarbozole, the labeled neurons could not be clearly focused under the oil immersion objective, thus precluding accurate cell measurements. However, they did not differ from the populations described above in the number of visible dendrites (see Table 6; Chi square, n.s.).

The results reported above were replicated in two hamsters processed for the immunofluorescent localization of neuropeptide Y immunoreactivity and retrograde transport of true blue. The true blue
injection sites were centered in the SCN in both cases. Both animals had many double-labeled neurons throughout the IGL and the anterior vLGNei (see Figure 15).

III.C. Experiment 4: Location of and retinal input to geniculate neurons immunoreactive to neuropeptide Y

This second experiment was designed to provide detailed information about the neuropeptide Y-immunoreactive neurons in the geniculate area. The first experiment in this chapter showed that neuropeptide Y-immunoreactive neurons make up at least part of the GST. Here I will describe the location of these neurons in the hamster brain. I will then describe material in which one can visualize both the pattern of retinal afferents and the neuropeptide Y-immunoreactive neurons in the geniculate, showing that different groups of neuropeptide Y-immunoreactive neurons receive different patterns of retinal innervation.

III.C.2.a. Methods

Several hamsters received unilateral intraocular injections of either wheat germ agglutinin-horseradish
peroxidase (WGA-HRP) (4 µl of a 1% solution in sterile saline; Sigma) or succinyl concanavalin A (10 µl of a 1% solution in distilled water; U.S. Biochemical). Two hamsters were used in each group. Animals were given an intraventricular injection of colchicine 48 hours later (see section III.B.1.a.) and sacrificed with a lethal dose of sodium pentobarbital 24 hours later. Perfusion and cutting were carried out as described in section III.B.1.a. Tissue was processed for WGA-HRP using the tetramethyl benzidine (TMB) reaction (Mesulam, 1978). In alternate sections, the TMB reaction product was stabilized using the method of Rye et al. (1984), and neuropeptide Y was subsequently visualized using immunocytochemistry, as described in section III.B.1.a. The anterograde transport of succinyl concanavalin A was visualized using immunocytochemistry. In one case the tissue was incubated overnight in both neuropeptide Y antibody (1:3750) and biotinated concanavalin A antibody (Vector Labs; diluted 1 µg/ml). The concanavalin A was developed using the Vectastain kit and avidin-biotin procedure (Hsu et al., 1981) with diaminobenzidine as a chromagen, after which the neuropeptide Y was developed using the peroxidase-anti-peroxidase procedure (Sternberger, 1979) and 3-amino-9-ethylcarbazole as a chromagen. In the other case, alternate sections of tissue were incubated
in neuropeptide Y antibody or rabbit concanavalin A antibody (U.S. Biochem; diluted 1:5000) and these were developed with the Vectastain kit using the avidin-biotin method (Hsu et al., 1981).

III.C 2.b. Results

Visualization of neuropeptide Y-immunoreactive cells in the geniculate area was markedly enhanced by colchicine treatment. The higher dose (200 μg) of colchicine was noticeably more effective than the 60 μg dose. The intergeniculate leaflet contained many neuropeptide Y-immunoreactive cells (see Figure 16). In the most caudal sections, this cell group was observed lying directly ventral to the medial geniculate body, extending into the zona incerta (see Figure 17). Most cells appeared elongated in frontal sections, with their long axes oriented laterally across the intergeniculate leaflet. Neuropeptide Y-immunoreactive cells were also observed in the anterior portion of the vLGNei (see Figure 18). At the rostral tip of the intergeniculate leaflet, scattered neuropeptide Y-immunoreactive neurons were observed along both the medial and lateral edges of the external division of the vLGN.

When alternate sections were processed for anterograde labeling of retinal terminal fields, all neuropeptide Y-immunoreactive cells in the
intergeniculate leaflet, both ipsilateral and contralateral to the injected eye, were found in areas receiving retinal input. By contrast, most neuropeptide Y-immunoreactive cells in the vLGNei were in an area of termination of fibers mainly from the contralateral eye; many immunoreactive cells ipsilateral to the eye injection were outside of labeled areas (see Figure 19). Results were similar whether WGA-HRP or succinyl concanavalin A was used as the tracer. These results were confirmed by examination of tissue with both retinal terminal fields and neuropeptide Y-immunoreactive cells labeled in the same section. In this material, the density of staining of the retinal terminal fields by WGA-HRP was somewhat reduced relative to the alternate sections which were not processed for neuropeptide Y-immunoreactivity.

III.D: Discussion

These experiments showed (1) that neuropeptide Y immunoreactivity is decreased in the SCN after ablation of the geniculate, (2) that geniculate neurons may be double-labeled for neuropeptide Y immunoreactivity and for a retrograde tracer transported from the SCN, (3) that neuropeptide Y neurons are located in the intergeniculate leaflet and in the anterior vLGNei, and
that the pattern of retinal afferents overlying neuropeptide Y-immunoreactive neurons in the IGL differs from that over neuropeptide Y-immunoreactive neurons in the anterior vLGN.

I have demonstrated that the intergeniculate leaflet of the hamster contains many neuropeptide Y-immunoreactive cells and that destruction of this area causes a reduction of neuropeptide Y-immunoreactive fibers in the suprachiasmatic nucleus. Thus, the geniculo-suprachiasmatic tract in the hamster appears to be similar to that described in the rat in that neuropeptide Y (or an immunoreactively similar peptide) is contained in at least a portion of these fibers (Card and Moore, 1982). Unilateral lesions of the intergeniculate leaflet and vLGN resulted in a reduction of neuropeptide Y immunoreactivity in both suprachiasmatic nuclei, with the suprachiasmatic nucleus ipsilateral to the lesion showing a greater relative reduction. This is in agreement with Pickard's (1982) report that 2 - 3 times more labeled cells appear in the ipsilateral intergeniculate leaflet after HRP injections in the suprachiasmatic nucleus, and with similar results in rats (Card and Moore, 1982).

The finding that destruction of the lateral geniculate area did not totally abolish neuropeptide Y immunoreactivity in the suprachiasmatic nucleus may
indicate that neuropeptide Y-immunoreactive cells from another brain area also project to the suprachiasmatic nucleus. Depletion of neuropeptide Y-immunoreactive fibers in the rat's suprachiasmatic nucleus after knife cuts of the stria terminalis has recently been reported (Allen et al., 1984). Combined with the observation of neuropeptide Y-immunoreactive cells in the amygdaloid complex, these results indicate that cells in the amygdala may supply a small portion of the neuropeptide Y-immunoreactive fibers observed in the suprachiasmatic nucleus.

Direct evidence that at least some of the geniculate neurons afferent to the hamster SCN contain neuropeptide Y was provided by double-labeling experiments. Double-labeled neurons were found in both the IGL and the anterior vLGNei. Neurons labeled with neuropeptide Y-immunoreactivity or WGA-HRP transported from the SCN were indistinguishable on the basis of maximum soma diameter, cell body area, orientation, number of dendrites and location. These observations indicate either that all geniculate neurons that project to the SCN contain neuropeptide Y or that all neurons projecting to the SCN and all neurons immunoreactive for neuropeptide Y are morphologically similar, but incompletely overlapping populations. These two possibilities cannot be distinguished based on the
present material.

The geniculo-suprachiasmatic tract of hamsters differs from that of rats in that a much greater proportion of the hamster suprachiasmatic nucleus contains neuropeptide Y-immunoreactive fibers (Card and Moore, 1982). This difference resembles that between the retinal terminal fields in the suprachiasmatic nucleus of these two species. In the rat, retinal afferents are confined to the ventrolateral suprachiasmatic nucleus (Card and Moore, 1982; Moore and Lenn, 1972), while retinal afferents reach most of the hamster suprachiasmatic nucleus, sparing only the dorsomedial area in the caudal two-thirds of the suprachiasmatic nucleus (Card and Moore, 1984; Pickard and Silverman, 1981).

Neuropeptide Y-immunoreactive cells in the intergeniculate leaflet lie in an area which receives bilateral retinal input. These results are consistent with electrophysiological evidence that at least some cells in the area of the intergeniculate leaflet which project to the suprachiasmatic nucleus are photically responsive (Groos and Rusak, 1982; see chapter IV). Most neuropeptide Y-immunoreactive cells in the anterior VLGNei, on the other hand, are in an area where input from the contralateral eye dominates and input from the ipsilateral eye is distributed in sparse patches. The
neuropeptide Y-immunoreactive cell bodies observed in the anterior vLGNei in the present study did not seem to be preferentially localized to the small areas receiving bilateral retinal input. However, we cannot determine from our present light microscopic material whether neuropeptide Y-immunoreactive cells actually receive direct retinal input, nor can we be certain that cells lying outside of areas receiving bilateral retinal input do not have dendrites extending into such areas (e.g., Mounty et al., 1977).

This is the first description of neurons in the anterior vLGNei that project to the SCN of hamsters (cf, Pickard, 1982). At least some of these neurons were immunoreactive for neuropeptide Y. These neurons may not be functionally related to similarly immunoreactive neurons in the IGL. The two areas differ in retinal input; the vLGN receives mainly contralateral retinal input, while the IGL receives binocular retinal projections.

Further support for the hypothesis that the intergeniculate leaflet is best considered a region separate from the vLGN comes from a report that the intergeniculate leaflet of the rabbit may contain an additional representation of the visual field that is distinct from that of the vLGN (Holcombe and Guillery, 1984). The retinotopic order of this retinal projection
to the intergeniculate leaflet was not precise and appeared to involve the complete overlap of ipsilateral and contralateral retinal input. These results are consistent with a role for the intergeniculate leaflet in entrainment of circadian rhythms, in that neither precise retinotopic order nor separation of input from the two eyes is logically necessary for this function. In addition, while there are no reported cytoarchitectural differences between the rostral and caudal vLGNe, there are many differences between the vLGNe in general and the IGL (Niimi et al., 1963; Swanson et al., 1974).

The present results, however, indicate that neurons immunoreactive for neuropeptide Y in the anterior vLGNei do project to the SCN, and that they are morphologically similar to those in the IGL. A functional similarity between these structures is also indicated by the finding that electrical stimulation in the anterior vLGNei produced phase shifts in hamster activity rhythms that were similar to those produced by IGL stimulation (Meijer et al., 1984). Afferents to the vLGNe from cortical area 17 appear to selectively spare the IGL and the anterior vLGNei (Schober and Gruschka, 1983). These observations suggest a close connection between the IGL and the anterior vLGNei, but it is still ambiguous whether the IGL and anterior vLGNei should be considered
a single functional unit.

In summary, this study has demonstrated that (1) at least some of the neuropeptide Y-immunoreactive cells in the area of the intergeniculate leaflet and vLGN appear to project bilaterally to the suprachiasmatic nucleus, sending more neuropeptide Y fibers to the ipsilateral than to the contralateral side; (2) the hamster intergeniculate leaflet contains cells immunoreactive for neuropeptide Y which lie in an area receiving bilateral retinal input, and (3) neuropeptide Y-immunoreactive cells that project to the SCN are also found in the external division of the anterior ventral lateral geniculate nucleus and many of these cells are in areas receiving only contralateral retinal input.
Figure 10. Cytoarchitectural divisions of the hamster lateral geniculate area. Three frontal sections of the nucleus are shown, with the topmost section being the most rostral. Abbreviations: DLGN = dorsal lateral geniculate nucleus, IGL = intergeniculate leaflet, OT = optic tract, ee = external part of the external division of the ventral lateral geniculate nucleus (vLGN), ei = internal part of the external division of the vLGN, i = internal division of the vLGN. (After Frost, So and Schneider, 1979; magnification = 44 X.).
Figure 11. Dark field photomicrographs of neuropeptide Y-immunoreactivity in the rostral (upper photos) and middle (lower photos) suprachiasmatic nucleus: (A) Control hamster. (B) Hamster sacrificed 28 days after a bilateral geniculate lesion. Note that a small number of neuropeptide Y-immunoreactive fibers are still observed at middle levels of the suprachiasmatic nucleus. (C) Hamster sacrificed 30 days after unilateral geniculate lesion. The left side of the photograph shows a decrease in neuropeptide Y-immunoreactivity in the suprachiasmatic nucleus ipsilateral to the geniculate lesion relative to the amount of immunoreactivity in the contralateral nucleus. (Magnification = 82 X.)
Figure 12: A representative lesion destroying the intergeniculate leaflet (IGL) and the ventral lateral geniculate nucleus (VLGN). Shading indicates the area damaged, which includes portions of the dorsal lateral geniculate nucleus (DLGN), medial geniculate nucleus (MGN), ventral thalamic nucleus and zona incerta. (Magnification = 17.5 X.)
Figure 13. Frontal brain section showing the center of the WGA-HRP injection site for one hamster (TMB reaction). The track of the micropipette (marked by an asterisk) parallel to the third ventricle (VIII) may be seen. This injection was mainly unilateral. Some involvement of the fibers of the supraoptic commissure (SOC) was noted. The suprachiasmatic nucleus (SCN) contralateral to the injection site is outlined by a dashed line. (Magnification = 195 X)
Figure 14. Distribution of cross-sectional area (μm²) for geniculate neurons labeled with either (A) neuropeptide Y-immunoreactivity (n = 69) or (B) WGA-HRP transported from the SCN (n = 105). Neuronal soma area was measured in one frontal section per neuron.
A. NPY

PERCENT OF CELLS

B. WGA-HRP

CROSS-SECTIONAL AREA (μm²)
Figure 15 Examples of double-labeled neurons in frontal sections through the geniculate area. (A.) A neuron in the anterior vLGNei labeled for neuropeptide Y-immunoreactivity with 3-amino-9-ethylcarbazole as the (red) chromagen. WGA-HRP transported from the SCN is visible as a black granular reaction product. (B.) The same neuron as in (A.), viewed through a 490 nm filter. The black WGA-HRP reaction product is clearly visible. (C.) Neurons in the intergeniculate leaflet labeled for neuropeptide Y-immunoreactivity with fluorescein. (D.) The same neurons as seen in (C.), labeled with true blue transported from the SCN. (E.) Neurons in the caudal intergeniculate leaflet labeled for neuropeptide Y-immunoreactivity with fluorescein. (F.) The same neurons as seen in (E.). One neuron is labeled with true blue transported from the SCN, while a second neuron (see arrow) is not labeled with true blue. Medial is to the left in A and B, and to the right in C through F.
Figure 16. Neuropeptide Y-immunoreactive cells and fibers in the intergeniculate leaflet at approximately the middle of the rostro-caudal extent of this nucleus in a colchicine-treated animal. (Abbreviations: DLGN = dorsal lateral geniculate nucleus, IGL = intergeniculate leaflet, OT = optic tract, VLGN = ventral lateral geniculate nucleus. Magnification = 440 X.)
Figure 17 Neuropeptide Y-immunoreactive cells and fibers in the caudal intergeniculate leaflet in a colchicine-treated animal. Note that these cells extend into the adjacent zona incerta. (Abbreviations: IGL = intergeniculate leaflet, MGN = medial geniculate nucleus, ZI = zona incerta. Magnification = 440 X.)
Figure 18. Neuropeptide Y-immunoreactive cells and fibers in the external lamina of the anterior ventral lateral geniculate nucleus (VLGN) in a colchicine-treated animal. (Abbreviations: DLGN = dorsal lateral geniculate nucleus, OT = optic tract, VLGNei = internal part of the external division of the VLGN, VLGNiv = ventral part of the internal division of the VLGN. Magnification = 440 X.)
Figure 19. Overlap of retinal terminal fields and neuropeptide Y-immunoreactive cells and fibers in the lateral geniculate area. Sections are arranged from rostral (upper) to caudal (lower). (A) Retinal terminal fields contralateral to the injected eye. (B) Retinal terminal fields ipsilateral to the injected eye. Note that a light ipsilateral innervation (lighter stippling) is observed over the intergeniculate leaflet and parts of the ventral lateral geniculate nucleus (VLGN); however, a patch of denser ipsilateral input (darker stippling) is seen in the VLGN at all levels. (C) Neuropeptide Y-immunoreactive cells and fibers. In the most rostral section, these cells are confined to the anterior external division of the VLGN (upper drawing) and many lie in areas receiving retinal input from only the contralateral eye. In more caudal sections, when the cells and fibers are found in the intergeniculate leaflet, binocular retinal terminal fields are found over all of the cells. (Abbreviations: DLGN = dorsal lateral geniculate nucleus, MGN = medial geniculate nucleus. Magnification = 22 X.)
Table 1. Classification scheme for vLGN and IGL neurons proposed by Brauer et al. (1984). Distinguishing characteristics for each neuron class are listed, along with subdivisions of the ventral geniculate in which that cell class may be found.
Table 1

<table>
<thead>
<tr>
<th>TYPE</th>
<th>DISTINGUISHING CHARACTERISTICS</th>
<th>AREA FOUND IN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>very large</td>
<td>vLGNei</td>
</tr>
<tr>
<td></td>
<td>(mean diameter = 17.7 um)</td>
<td>vLGNee</td>
</tr>
<tr>
<td></td>
<td>- proximal dendrites can be seen in Nissl stained material</td>
<td>IGL</td>
</tr>
<tr>
<td>Class 2</td>
<td>largest vLGN neurons (&gt; 20 um diameter)</td>
<td>vLGNei</td>
</tr>
<tr>
<td></td>
<td>- scarcely distributed</td>
<td>vLGNee</td>
</tr>
<tr>
<td></td>
<td>- cannot see proximal dendrites in Nissl stained material</td>
<td>vLGNiv</td>
</tr>
<tr>
<td>Class 3</td>
<td>medium-sized</td>
<td>IGL</td>
</tr>
<tr>
<td></td>
<td>(mean diameter = 14.9 um)</td>
<td>vLGNiv</td>
</tr>
<tr>
<td></td>
<td>- short dendrites</td>
<td>vLGNei</td>
</tr>
<tr>
<td></td>
<td>- oval, with a moderate amount of cytoplasm if Nissl stain</td>
<td>vLGNee</td>
</tr>
<tr>
<td>Class 4</td>
<td>elongated soma</td>
<td>IGL</td>
</tr>
<tr>
<td></td>
<td>- polarized dendrites</td>
<td>vLGNiv</td>
</tr>
<tr>
<td></td>
<td>- cytoplasm concentrated at poles</td>
<td></td>
</tr>
<tr>
<td>Class 5</td>
<td>dendrites have very thin branches, resembling axons</td>
<td>IGL</td>
</tr>
<tr>
<td></td>
<td>- can not identify</td>
<td>vLGNiv</td>
</tr>
<tr>
<td></td>
<td>- in Nissl stained material</td>
<td>vLGNei</td>
</tr>
</tbody>
</table>

**Note:** The text appears to be a transcription error or a mix-up in formatting, as it contains both vertical and horizontal lines, making it difficult to read. The content seems to be scientific or medical in nature, possibly discussing cell types or neuron characteristics.
Table 2. Afferent projections of the vLGN and IGL as described in several survey papers. The "+" in each column indicates that that particular reference describes such a projection. References R1 to R3 refer to the vLGN of the rat, while references C1 and C2 refer to the vLGN of the cat. References are: R1. Cosenza and Moore, 1984; R2. Pasquier and Villar, 1982a; R3. Mackay-Sim et al., 1983; C1. Hughes et al., 1981; C2. Graybiel, 1974.

Abbreviations: LP, lateral posterior thalamic nucleus; MRF, mesencephalic reticular formation.
### Table 2

<table>
<thead>
<tr>
<th>AREA</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Cl</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral vLGN</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lateral terminal nucleus</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pretectum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zone incerta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subthalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perirubral fields</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central gray</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual cortex</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dorsal raphe</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRF</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periaqueductal gray</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dorsal tegmental nucleus</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parabigeminal nucleus</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vestibular nuclei</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Efferent projections of the vLGN and IGL as described in several survey papers. The "+" in each column indicates that that particular reference describes such a projection. References R1 through R4 refer to the vLGN of the rat, while references C1 through C3 refer to the vLGN of the cat. References are: R1: Legg, 1979; R2. Ribak and Peters, 1975; R3. Graybiel, 1974; R4. Swanson et al., 1974; C1. Graybiel, 1974; C2. Edwards et al., 1974; C3: Swanson et al., 1974. Abbreviations: LTN, lateral terminal nucleus; MTN, medial terminal nucleus; SCN, suprachiasmatic nucleus.
<table>
<thead>
<tr>
<th>AREA</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contralateral vLGN</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>LTN</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pons</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Zona incerta</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>SCN</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTN</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subthalamus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perirubral fields</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central gray</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Distribution of retinal terminal fields of various mammals within the subdivisions of the vLGN. "B" indicates input from both eyes, while "C" indicates input from the contralateral eye only and "0" indicates no retinal input to this area. The "-" indicates that the authors did not make an explicit statement regarding the retinal terminals in this division. The references and the animal studied are: A. Swanson et al., 1974; rat; B. Hickey and Spear, 1976; rat; C. Frost et al., 1979; hamster; D. Kicliter and Bruce, 1983; 13-lined ground squirrel; E. Takahashi et al., 1977; rabbit; F. Holcombe and Guillery, 1984; rabbit.
<table>
<thead>
<tr>
<th>SUBDIVISION</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>vLGNe</td>
<td>C, B, B, B, B, B, B</td>
</tr>
<tr>
<td>vLGNei</td>
<td>- , C, B</td>
</tr>
<tr>
<td>vLGNe</td>
<td>- , B, - , B</td>
</tr>
<tr>
<td>vLGNi</td>
<td>- , C, U, B, C, C</td>
</tr>
<tr>
<td>IGL</td>
<td>- , B, B, B, B, B, B</td>
</tr>
</tbody>
</table>
Table 5. The amino acid sequences of neuropeptide Y (NPY), avian pancreatic polypeptide (APP) and molluscan cardioexcitatory peptide (FMRF).

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY</td>
<td>YPSKPDNGEDAPAEALARYSSALHRTINLITRQRY-NH2</td>
</tr>
<tr>
<td>APP</td>
<td>GPSQPTYPGDDAPVEDLIRFYDNQLQYLNVTNHRY-NH2</td>
</tr>
<tr>
<td>FMRF</td>
<td>FMRF-NH2</td>
</tr>
</tbody>
</table>
Table 6. Percent of geniculate neurons in one hamster with one, two or more than two dendrites visible in one frontal section. Neurons were labeled either with neuropeptide Y-immunoreactivity (NPY; \( n = 69 \)), WGA-HRP transported from the SCN (\( n = 105 \)), or both (double-labeled; \( n = 119 \)).

<table>
<thead>
<tr>
<th>No. of dendrites</th>
<th>NPY</th>
<th>WGA-HRP</th>
<th>Double-labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15%</td>
<td>9%</td>
<td>19%</td>
</tr>
<tr>
<td>2</td>
<td>68%</td>
<td>69%</td>
<td>69%</td>
</tr>
<tr>
<td>&gt;2</td>
<td>17%</td>
<td>22%</td>
<td>12%</td>
</tr>
</tbody>
</table>
CHAPTER IV. Extracellularly recorded responses of GST neurons to whole eye illumination

IV.A. Introduction and literature review

This chapter will describe the responses of geniculate neurons to diffuse whole eye illumination in hamsters. Response characteristics of neurons will be separately analyzed for the IGL and for each of the subdivisions of the vLGN. We know from the anatomical experiments of chapter III that the geniculate neurons likely to be projecting to the SCN are located in the rostral vLGN and in the IGL. The responses of neurons in these areas will be contrasted to those in other parts of the vLGN. Responses of geniculate neurons antidromically activated by SCN stimulation will be described. The responses of geniculate neurons to varying light intensities will be reported, and luminance thresholds of putative GST neurons will be compared to those of SCN neurons.

As an introduction to these experiments, I will first review previous work on the visual responses of vLGN neurons. The second section of the introduction will briefly describe previous electrophysiological studies of the mammalian circadian system and the third
section will describe previous studies of the physiological effects of neuropeptide Y, a putative neurotransmitter of the GST. The final section will introduce the experiments to follow.

IV.A.1. Electrophysiological recordings of unit activity in the vLGN

Results from surveys of single unit recordings in the vLGN have been reported for the rat, rabbit and cat. None of these reports differentiated among the subdivisions of the vLGN. Thus it cannot be determined to what degree the results of these studies reflect the activity of GST neurons; in fact, it is not known if the rabbit or cat even have a geniculó-suprachiasmatic tract. For this reason, I will only summarize major results and will not review these studies in detail here. A detailed review is, however, contained in Appendix B.

In general, the vLGN may be distinguished from surrounding retinorecipient nuclei in several respects. Retinal input appears to originate mainly from retinal ganglion cells with relatively slow conduction velocities (W-cells; Stone and Fukuda, 1974). Some units appear to code luminance levels by their level of maintained discharge. At least in the cat, some vLGN units are responsive to vestibular and optokinetic
stimulation.

It appears that there is a good deal of variation in visual receptive field characteristics of vLGN units across species, although in some instances even more variation exists across studies using the same species. I will indicate the major species differences in the remainder of this section. Table 7 summarizes the results of all the studies surveying receptive field characteristics of vLGN units in the rat, rabbit and cat.

Results from surveys of unit activity in the rat vLGN differ considerably (see Appendix B.1.). The earliest study (Hale and Sefton, 1978) may contain the most representative sample of vLGN units. The two later studies (Hayashi and Nagata, 1981; Sumitomo et al., 1979) did not use a histological criterion, but accepted a unit as a vLGN unit only if it responded to stimulation of both the optic chiasm and the visual cortex. This criterion may have led to selectively excluding units which do not receive visual cortical input. This class of units is probably concentrated in the areas relevant to this thesis, the IGL and the rostral vLGN (see Schober, 1983). One conclusion from Hale and Sefton's study is that the rat vLGN contains about 3 times as many sustained "on" units as phasic units. Many of these sustained "on" units increase
their firing rate with increases in luminance levels. Units in the rat vLGN are slightly more likely to have a concentric than a uniform receptive field organization (although the results of Sumitomo et al., 1979, differ dramatically from this generalization). Most vLGN units in the sample of Sumitomo et al. (1979) responded to illumination of the contralateral eye only. The one study of receptive field characteristics of units in the rabbit vLGN indicated that visual response properties of these units did not differ dramatically from those of units in the rat vLGN (see Appendix B2).

Visual response characteristics of units in the cat vLGN appear to be quite different from those in the rat or rabbit vLGN. A smaller proportion of the units in the cat vLGN have sustained "on" responses or firing rates that tonically increase or decrease in response to changes in luminance levels, while a greater proportion of units have phasic responses to light (see Table 7). It appears that units with uniform receptive fields always predominate over those with a concentric organization. A few vLGN units with active surround regions have been described in the cat (Spear et al., 1977), while only silent surrounds are found in samples of units in the rat vLGN. As opposed to the rat vLGN where responses are mainly contralaterally driven, many units of the cat vLGN respond to photic stimulation of
both eye's, and some studies find a number of units responding solely to the ipsilateral eye. Wavelength-sensitive vLGN units have been described only in the cat (Hughes and Chi, 1983). The rat, rabbit and cat all seem fairly similar in the percentage of movement sensitive units.

It appears likely that the visuotopic organization of the vLGN of the cat and the rat differ (Hada et al., 1986; Nagata and Hayashi, 1984) but the visuotopic organization of the cat vLGN has not been studied in detail. In the rat, most visually responsive units are found in the vLGNb. The rabbit vLGN is quite different in this respect; many visually responsive units are found in the vLGNi as well as in the vLGNe. The cat vLGN resembles that of the rat in that visually responsive units are reported to be mainly in the lateral parts of the vLGN, but the vLGN of the cat differs from that of the rat or rabbit in that a fairly large proportion of the vLGN units in the cat have no clear visual response.

The medial part of the vLGN in the cat appears to contain units responsive to vestibular and optokinetic stimulation. Many of these units fire in synchrony with saccadic or nystagmic eye movements. Most of these units are unresponsive to photic stimulation. Such units have not been searched for in the vLGN of other
species, but it is possible that they are more common in the cat vLGN since more units unresponsive to visual stimulation are found in the cat vLGN than in the rat or rabbit vLGN.

The pregeniculate nucleus of the primate is thought to be the homologue to the vLGN. An early study of visual responses of units in this nucleus (DeValois, 1960) reported that many units responded to visual stimulation of either eye. All units gave sustained "on" responses. Later studies indicated that other units in this area respond to saccadic eye movements, but not to vestibular stimulation (Buttner and Fuchs, 1973; Magnin and Fuchs, 1977). Thus, responses of units in the primate pregeniculate nucleus appear to differ in some respects from those of vLGN units in other species.

In contrast to the studies discussed above, one study of vLGN units did identify GST neurons (Groos and Rusak, 1982). Of a total sample of 353 vLGN units in the rat, 12 units were antidromically activated by electrical stimulation of the SCN. Seven of these units showed sustained "on" responses to light, two units showed sustained "off" responses, one unit gave phasic responses and two units did not respond to retinal illumination. These results indicate that the majority of GST neurons in the rat show sustained "on" responses to illumination.
IV.A.2. Electrophysiological studies of the mammalian circadian system

The suprachiasmatic nuclei are the locus of the major pacemaker of the mammalian circadian system. The SCN of all mammals examined receives bilateral retinal input and single units recorded in the SCN of the rat, hamster, and cat have been demonstrated to be responsive to light (Groos and Mason, 1978; Lincoln, Church and Mason, 1975; Meijer et al., 1986). Binocular responses are observed in some visually responsive SCN neurons in the rat (Lincoln et al., 1975), but not in the cat (Groos and Mason, 1980). Single units in the rat SCN have been shown to respond orthodromically to electrical stimulation of the contralateral or ipsilateral optic nerve (Sawaki, 1979). Cahill (pers. comm.) has measured the conduction velocity of the retinal afferents to the SCN of the mouse using an in vitro technique; a brain slice with the optic nerves attached was prepared and responses of SCN neurons to electrical stimulation of the optic nerve were recorded. His preliminary results indicated that these retinal axons have extremely slow conduction velocities (approximately 0.6 m/s).

A maintained increase in illumination is a more optimal stimulus for SCN neurons than brief light
flashes (Meijer et al., 1986). All visually responsive neurons in the SCN appear to have sustained responses, although some units in the hamster and cat SCN also have a phasic component to their visual responses (Groos and Mason, 1980; Meijer et al., 1986). Sustained "on" units are approximately two to three times more common than sustained "off" (light suppressed) units in the rat and cat (Groos and Mason, 1978, 1980; Meijer et al., 1986; Sawaki, 1977) and approximately 5 times more common in the hamster SCN (Meijer et al., 1986). These two types of responses (activated and suppressed) can be recorded in an SCN slice preparation in response to optic nerve stimulation (Shibata et al., 1984), indicating that intact retinal ganglion cells are not necessary for the production of these opposing responses. Visual receptive fields of SCN units in the cat are typically very large, often greater than 20 degrees in diameter. All receptive fields studied in the cat SCN had a uniform organization; antagonistic surrounds were never observed (Groos and Mason, 1980).

Although the neurons in the SCN appear to generate a circadian rhythm in firing rate (Green and Gilette, 1982; Groos and Hendriks, 1982; Inouye and Kawamura, 1979; Shibata et al., 1982), the visual responsiveness of SCN units does not appear to vary in a circadian manner. This was shown in a study of multiple unit
activity recorded via electrodes chronically implanted in the SCN of a rat (Inouye, 1984). Knife cuts were used to surgically isolate the SCN from surrounding hypothalamic tissues, leaving the retinohypothalamic tract intact. Circadian rhythms of multiple unit activity could be recorded from a single animal. Activity which showed robust responsiveness to illumination was examined for circadian variation. There was no statistically significant circadian rhythm in visual responsiveness of the multiple unit activity recordings. This correlates with reports that visual responses recorded from single units in the SCN of the rat do not appear to differ with the projected circadian phase of the anesthetized animal (Groos and Mason, 1980; Meijer et al., 1986).

If the intensity of illumination is varied and the level of maintained discharge of a single unit in the SCN is calculated for each intensity, a monotonic intensity-response function is obtained. These curves typically have a threshold level below which the unit does not respond, a working range of approximately three log units, and saturation of the response at higher levels of illumination. Half-maximal responses are typically observed at light intensities of approximately 100 lux (20 μW/cm²) in the hamster and 10 lux in rat SCN neurons (Meijer et al., 1986). Threshold levels may
vary with the previously experienced illumination (Meijer et al., 1988), but these changes in threshold are thought to be of minor functional importance, since the intensity-response function is usually shifted by only one order of magnitude and the curvature is only slightly affected (Groos and Meijer, 1985).

Groos and Mason (1978) reported that visual responses were rare when recording from the SCN of rats 3 to 4 months after geniculate ablation, although both light-activated and light-suppressed responses could be recorded. These results indicated that ablation of the geniculate might affect visual responsiveness of SCN neurons; however, later reports did not confirm this conclusion. Sawaki (1979) reported that percentages of SCN neurons showing both activation and suppression to optic nerve electrical stimulation immediately after transection of the optic tracts posterior to the SCN did not differ from those in intact control rats. These results would appear to indicate no effect of the loss of GST input on responses of SCN neurons to optic nerve stimulation; however, the immediate effects of GST destruction might possibly be different from longer term effects. Groos and Rusak (1982) reported that ablation of the geniculate did not alter visual responsiveness of single units in the SCN of rats. This study was very similar to the initial study of Groos and Mason (1978)
and therefore indicates that the results of the earlier report may not be replicable.

In the study described earlier, involving chronic multiple unit recordings from rats with the SCN surgically isolated from all input except the retinohypothalamic tract, only light activated responses were observed (Inouye, 1984). These results should be treated cautiously since (1) the technique of multiple unit recordings necessarily involves sampling of a small number of grouped units in one small part of the SCN, and (2) the number of rats with multiple unit recordings showing light responsiveness was very small (five rats). The most likely conclusion from these studies appears to be that input from the GST is not necessary for visual responses of SCN neurons; however, it is possible that the visual responsiveness of SCN neurons is subtly affected by loss of geniculate input.

IV.A.3. Physiological actions of neuropeptide Y and related peptides

This section will review studies on the physiological actions of neuropeptide Y and related peptides. Only a few studies have characterized the physiological actions of neuropeptide Y on central nervous system neurons and none have reported on the
actions of neuropeptide Y on SCN neurons.

Neuropeptide Y is one of the most potent vasoconstrictor peptides that has been identified (Emson and DoQuidt, 1984). This vasoconstrictor effect is seen only on arteries and not on veins, in both the central and the peripheral nervous systems. The vasoconstrictor effect of neuropeptide Y appears to be calcium dependent since it can be blocked by reducing extracellular calcium (Edvinsson et al., 1983; cat cerebral arteries) or by administration of calcium antagonists (Pernow et al., 1986; rat femoral arteries). Neuropeptide Y has been shown to inhibit forskolin-stimulated cAMP accumulation in cerebral arteries of the cat (Fredholm et al., 1985). Neuropeptide Y appears to have both post- and presynaptic effects on vascular tissue. Neuropeptide Y applied alone can have vasoconstricting effects. Neuropeptide Y can also potentiate the effects of other vasoconstricting substances such as norepinephrine (Edvinsson et al., 1984) but does not affect stimulation-induced norepinephrine release. Therefore, in the vascular system, neuropeptide Y appears to act postsynaptically to cause vasoconstriction and presynaptically to potentiate the vasoconstricting effects of norepinephrine.

In the vas deferens, however, neuropeptide Y appears to have no postsynaptic effects; no effect of
neuropeptide Y application alone is seen. Application of neuropeptide Y does have presynaptic effects on rat vas deferens tissue, and this effect is opposite to that seen in vascular tissue in that an inhibition of norepinephrine release is observed (Lundberg et al., 1982).

As in the peripheral nervous system, neuropeptide Y appears to have central nervous system actions which vary depending upon the structure studied. Thus, neuropeptide Y appears to act presynaptically to reduce population spikes and EPSPs evoked by stratum radiatum stimulation in CA1 neurons in a hippocampal slice preparation (Colmers et al., 1985). Neurons in the CA1 layer show normal resting potentials, action potentials and input resistance when neuropeptide Y is perfused through the bath of the slice, indicating that there is no postsynaptic effect of the peptide. These effects are similar to those of neuropeptide Y on the vas deferens.

Day et al. (1985) recorded from putative vasopressin synthesizing neurosecretory neurons in the supraoptic nucleus of rats. Pressure-injected neuropeptide Y increased the firing rate of most of these neurons, indicating a probable postsynaptic excitatory effect of neuropeptide Y, similar to that seen in vascular tissue. Interestingly, APP showed much
more potent excitatory effects on these neurons than did neuropeptide Y. The effect of APP was similar in strength to that of norepinephrine. Co-application of APP, however, appeared to diminish the excitatory effect of norepinephrine. This inhibitory effect of APP on the response to norepinephrine lasted up to 120 s after the APP application, which is consistent with the relatively long time course generally posited for the actions of peptides.

In several identified neurons in the mollusc, Helix aspersa, FMRF-amide decreased calcium conductance (as neuropeptide Y does in the mammalian vascular system), indicating a presynaptic inhibitory effect (Colombaion et al., 1985). FMRF-amide may also affect potassium conductance in these neurons.

In summary, despite some observed consistencies, neuropeptide Y can have radically different physiological effects in different systems, and one cannot safely predict its activity in an unstudied structure. Neuropeptide Y can have both pre- and postsynaptic effects, which may be either inhibitory or excitatory. One study indicated that some effects of the related peptide APP may have a relatively long time course (Day et al., 1985). Although it is generally accepted that neuropeptide Y and not APP is endogenous to the mammalian central nervous system, several studies
indicate that APP may also be found in the mammalian central nervous system (Inui et al., 1985) and may have stronger effects on central neurons than those of neuropeptide Y (Dáy et al., 1985). It remains uncertain whether GST neurons contain only one or both of these peptides.

IV.A.4. Introduction to electrophysiological studies of the hamster GST

In this study, I characterized the visual responses of neurons in the hamster IGL and vLGN. To identify individual neurons forming the GST, a stimulating electrode was placed in the area of the SCN of some animals and GST neurons were antidromically activated. Thus, the visual responses of identified GST neurons could be characterized. Antidromic activation also provided confirmation of anatomical studies (see Chapter III) indicating that the projection from the geniculate to the SCN is monosynaptic.

One hypothesis tested in this study is that the GST (via release of neuropeptide Y) signals darkness to the circadian pacemaker in hamsters. This hypothesis is derived from the report of Albers and Ferris (1984) that injection of neuropeptide Y into the SCN results in phase shifts similar to those induced by dark pulses. If we assume that a high firing rate of GST neurons
leads to a high rate of release of neuropeptide Y in the SCN, then from Albers and Ferris' results we might expect a high firing rate of GST neurons to correspond with periods of darkness. This is supported by the report of Meijer et al. (1984) that electrical stimulation of the vLGN also results in phase shifts similar to those induced by dark pulses. There is good reason, however, to doubt this hypothesis. First, it has been shown that most of the neurons of the GST in rats are tonically activated by light, although tonically suppressed neurons are also observed (Gros and Rusak, 1982). Second, hamsters with GST ablation show differences in their circadian rhythms under L,L, but not under DD, when compared to control hamsters (see Chapter II). In this study, I will determine the visual response characteristics of GST neurons in hamsters.

A second hypothesis tested in this study was that GST input affects the luminance coding properties of SCN neurons. Since ablation of the GST causes only subtle changes in the entrainment of circadian rhythms of mammals (see section II.A.2), it might be hypothesized that the effect of GST neurons on the photic input to the SCN is a subtle one. Reports that GST ablation did not produce dramatic changes in SCN unit responses (e.g., loss of all "on" or "off" responses; see section IV.A.2) does not rule out the possibility that subtle
features of SGN photic responsiveness are modulated by GST input. The luminance-coding properties of GST neurons will be measured in this study, to allow comparison with those of hamster SCN neurons (Meijer et al., 1986). If GST input alters the luminance-coding properties of SCN neurons, then the luminance-response curves of GST neurons might be expected to differ from those of SCN neurons.

IV.B. Experiment 5: Visual responses of neurons in the ventral lateral geniculate area

IV.B.1. Methods

Forty-one male golden hamsters (Charles River; Lakeview, N.J.; 90 - 160 g) were housed in a colony room under a light-dark 14:10 cycle with lights on at either 0600, 2000 or 2200 h (Atlantic Daylight Time). Hamsters were anesthetized between 0800 and 1200 h with intraperitoneal injections of 20% urethane (2 g/kg body weight). Either 1% atropine sulphate (12 mg/kg body weight) or 0.02% Robinul (Robins Canada Ltd.; 0.3 mg/kg body weight) was injected subcutaneously to reduce fluid accumulation in the lungs. Supplementary doses of drugs were administered as necessary to maintain anesthesia and free breathing throughout the experiment. The
rectal temperature of the animal was monitored throughout the experiment and maintained at 37°C by a heating pad.

The hamster was positioned in a Kopf stereotaxic instrument with the incisor bar 2.0 mm below the interaural line. Extracellular single-unit recordings were made with either homemade, platinum iridium glass-coated electrodes (1.0 - 2.5 MΩ impedance at 1 kHz) or tungsten Parylene-coated electrodes (Microprobe, Inc.; 1 - 2 MΩ impedance at 1 kHz). The recording electrode was positioned over the area of the lateral geniculate nucleus and lowered by a microdrive (coordinates: 1.4 +/- 0.3 mm caudal to bregma; 3.4 +/- 0.4 mm lateral to the midline; 4.4 +/- 1.4 mm ventral from dura). In 17 animals, a concentric bipolar stimulating electrode was positioned over and lowered to the area of the suprachiasmatic nucleus (coordinates: 0.7 +/- 0.7 mm anterior to bregma; 0.1 +/- 0.1 mm lateral to the midline; 7.6 +/- 0.4 mm ventral from dura).

Amplification of the signal from the recording electrode was provided by a preamplifier with a remote probe which was placed close to the animal (Bak Electronics Inc., Model A-1). A window discriminator converted action potentials into electronic pulses which were then fed into an Apple II computer controlling
stimulus presentations. Two or more units at the same location could be distinguished on the basis of waveform, and the window discriminator could be set to record from only one unit. Both the action potentials and the output from the window discriminator could be observed on a storage oscilloscope. Only action potentials from cell bodies were studied. These were distinguished from action potentials of axons using the criteria of Bishop et al. (1962). After recording from a unit, the recording electrode was advanced until that unit could no longer be discriminated from the background noise. The next action potential recorded was assumed to be from a different cell.

Stimulation was provided by a Grass S44 stimulator. To reduce the stimulus artifact, a stimulus isolation unit (Grass SIU5) was connected in series with the stimulator. A constant current unit (Grass CCU1A) allowed control of stimulus current. Biphasic pulses of 0.4 - 1.0 mA, 0.1 - 0.25 ms were used to search for responses. When a response was found, the threshold current was determined and a pulse of twice the threshold was commonly used for further testing. Responses to stimulation were photographed directly from the oscilloscope. Criteria for antidromic activation were: 1) constant latency of response (variation of less than 0.1 ms to suprathreshold stimulation), 2) following
of high frequency stimulation (up to 100 Hz), and 3) a successful collision test (Fuller and Schlag, 1976). The collision test was the essential criterion for a response to be classified as antidromic. Orthodromic responses elicited by stimulation of the optic chiasm directly ventral to the SCN were generally more variable in latency, did not follow high frequency stimulation, and did not pass the collision test.

A tungsten halogen incandescent light (Sylvania EKE, 150 W, 21 V) was used for photic stimulation. The light was led by a glass fiber light guide (Bolan-Jenner, Inc.) to a series of lenses, an infrared filter and a computer-controlled electronic shutter.

Neutral density filters could be positioned in front of the shutter to control the intensity of illumination.

In the majority of the experiments, a piece of diffusing glass was placed in front of each of the hamster's eyes. A solution of 1% atropine sulphate was used to keep the hamster's pupils maximally dilated. Mineral oil was used to keep both the eyes and the exposed cortical surface moist. All cells were tested for their responses to photic stimulation of the contralateral eye. In some cases, responses to binocularly or ipsilaterally applied photic stimulation were also tested. Light presentations ranged from less than 1 s to 45 min in duration; presentations of 60 s were most
common. Responses to repeated presentations were summed by the computer for signal averaging. A raster display allowed examination of the response to each stimulus presentation. A unit that was classified as visually unresponsive was only included in the sample if visually responsive units were recorded on that electrode track both before and after that unit.

Locations of all visually responsive units were determined histologically. At the site of a particularly interesting unit and at the site of the last unit recorded, the position of the recording electrode was marked with a small electrolytic lesion by passing 5 - 10 μA D.C. (cathodal) through the electrode for 6 - 10 s. A second lesion was usually made 1 mm below the last unit recorded to allow later correction for tissue shrinkage during histological processing. Animals were perfused intracardially with physiological saline followed by 10% formalin. Brains were placed overnight in a 10% sucrose-formalin solution after having been postfixed for several days. Brain sections (40 μm) were cut on a cryostat, mounted on chrome-alum coated slides and stained by the Kluver-Barrera method (Kluver and Barrera, 1953).

IV.B.2. Results
Histological reconstructions of recording electrode tracks (see Figure 20) indicated that 73 units in the intergeniculate leaflet and 197 units in the vLGN were tested for visual responses. Units in the vLGN could be further classified by the subdivision of the vLGN in which they were located (see section III.A.1). Units in the anterior third of the vLGN were distinguished from units in the remainder of the vLGN (see section III.D).

IV.B.2.a. Survey of visual responses

Visual responses were classified into seven categories. "Sustained on" units were those that showed a tonic firing rate during visual stimulation that was higher than the tonic firing rate during darkness. All these units maintained this high firing rate for the total time of visual stimulation (a minimum of 60 s). "Sustained off" units showed the opposite response of a maintained high firing rate during darkness and a lower tonic firing rate during the light stimulation. "Long on" units showed an increase in firing rate when the eye was illuminated which was maintained for at least 20 s but not as long as 60 s. "On" and "off" units gave a brief burst of firing correlated with the visual stimulus onset or end respectively, returning to their baseline firing rate within 5 s. "On/off" units gave
similarly phasic responses, but responded to both the onset and the end of the visual stimulus.

Visual responses of IGL units differed from those of vLGN units (Chi-square test, p < .001). Visual responses in the IGL were mainly "sustained on" type (see Table 8). While 68% of IGL units gave "sustained on" responses to light, only 29% of vLGN units gave similar responses. Of 50 "sustained on" IGL units, 30 units had an initial phasic response to light onset. Two units also had a phasic response at the end of the light presentation. The "sustained on" IGL units showed a maintained high firing rate during continuous bright illumination for up to 1 hour (see Figure 21). The average (± s.d.) sustained firing rate in darkness of these units was 3.0 ± 3.7 spikes/s. Sustained firing rates during contralateral eye illumination (usually measured during the second 30 s of a 60 s light presentation) were as high as 39 spikes/s (mean ± s.d. = 11.0 ± 10.0 spikes/s) and were often unusually regular in their discharge pattern. Phasic responses of "sustained on" IGL units involved peak firing rates of up to 61 spikes/s. Four IGL units showed oscillatory firing patterns in darkness. These oscillations were often seen immediately after a long light presentation. Each unit oscillated with a different frequency (0.013 Hz, 0.004 Hz, 0.002 Hz, and 0.11 Hz) but each unit's
frequency was consistent over repeated trials.

Units in the vLGN were more likely to give phasic responses to whole eye illumination than were IGL units (Chi square test, \( p < .001 \)). Phasic visual responses were observed in 46\% of vLGN units and 8\% of IGL units. The vLGNiv was the only subdivision of the vLGN in which visual responses of units were significantly different from responses in the remainder of vLGN units (Chi square test, \( p < .001 \)). A greater percentage of "on/off" responses were observed in the sample of vLGNiv units than in the remainder of the vLGN (Chi square test, \( p < .001 \)). There were no apparent differences between visual responses of units sampled during the projected light and dark periods of the hamster's housing condition.

Units for which no visual response could be determined were found in the vLGNiv \((n = 7)\) and the anterior vLGNei \((n = 1)\). These units were included in the classification "other" in Table 8. The remaining cells classed as "other" had a variety of responses. The response of a phasic excitation at "on" coupled with a brief inhibition at "off" was fairly common (10 units; 7 in the vLGNei). Four units, two of which were in the IGL, showed the opposite response ("off" excitation and "on" inhibition). Nine units showed responses of inhibition at "on", "off" or both. The remaining six
units classed as "other" had inconsistent visual responses.

In summary, IGL units showed mostly "sustained on" visual responses. vLGN units showed both sustained and phasic responses, with the phasic responses predominating. Units in the anterior one-third of the vLGN appeared similar to those in the remainder of the vLGN with respect to their responses to illumination. Visual responses of units in the vLGN differed from those in the rest of the vLGN.

Eleven of sixteen IGL units tested showed responses to illumination of the ipsilateral eye (see Table 9). Nine of these units were inhibited in response to illumination of the ipsilateral eye. Seven of these nine units had "sustained on", while two had "sustained off" responses to contralateral visual stimulation (see Table 10). Of the nine ipsilaterally suppressed units, seven were tested for responses to binocular illumination. Four of these seven units appeared to show weaker transient responses to binocular illumination than were seen in response to illumination of the contralateral eye alone (see Figure 22). The five IGL units that did not respond to ipsilateral illumination had "sustained on" responses to illumination of the contralateral eye.

Thirty-six vLGN units were tested for binocular
visual responses. Of these 36 units, only 10 showed
responses to illumination of the ipsilateral eye; 7 of
these 10 were located in the anterior vLGNei and all
were in some part of the vLGNei (see Table 9). Three of
these ten units showed similar responses to both
ipsilateral and contralateral eye illumination, although
the response to ipsilateral illumination was less
robust. There was a tendency, similar to that seen in
the IGL, for "sustained on" units to decrease firing
rate in response to illumination of the ipsilateral eye
(see Table 10).

The percent of binocularly driven units in the IGL
was not different from that in the anterior vLGNei
(Fischer's exact test, n.s.). Anterior vLGNei units
differed from remaining vLGNei units sampled in the
amount of binocularity observed (Fischer's exact test, p
< 0.05, see Table 9).

Several "sustained on" units were tested for their
responses to a range of light intensity levels. The
four IGL units tested showed an increase in their firing
rate with increased light intensity (see Fig. 23). A
half maximal response was observed at light intensities
of approximately 35 \( \mu \text{W/cm}^2 \) for most units. The firing
rates of all units increased in a graded manner as light
intensity increased, until saturation of the response at
intensities around 300 \( \mu \text{W/cm}^2 \). One unit appeared to
decrease its firing rate as light intensities increased beyond 300 μW/cm² (see Fig. 23c).

Light intensity response curves of two "sustained on" units in the anterior vLGNei did not appear to differ from those of IGL units (see Fig. 24). One dLGN "sustained on" unit for which a light intensity response curve was measured did not appear to show graded increases in firing rate with increased light intensity (see Fig. 25a), while another dLGN unit may have had such a response (see Fig. 25b). This unit was tested differently from all the others in that light intensity was changed in gradually increasing and decreasing steps, with no intervening periods of darkness.

One IGL unit and one vLGNei unit were tested for responses as light intensity was first decreased and then increased. Sixty second light presentations were alternated with 60 s of darkness and several measures were taken at each light intensity level before changing the level. When light intensities were increased it appeared that these cells saturated at lower intensity levels than they did when light intensities were decreased (see Fig. 23b, 24a). It appears that these cells have a larger working range for signalling decreasing than increasing light intensities.

IV.B.2.b. Stimulation of the SCN area
Two units, both located in the IGL, were antidromically activated by SCN stimulation. In one case, the stimulating electrode was in the caudal SCN contralateral to the recording electrode. The threshold current for the unit's response was 0.4 mA for a stimulation pulse of 0.25 ms duration. As current was increased to twice the threshold current, latency of the response decreased by approximately 0.2 msec. The response was seen at a constant latency (4.4 ms at twice-threshold stimulation) and could follow stimulation at rates up to 175 Hz. A collision test indicated that this was a true antidromic response. A collision of the stimulation-induced action potential with the spontaneous action potential was seen when the stimulation followed the spontaneous spike by 3 ms or less (see Figure 26). Double-pulse stimulation indicated that the refractory period of this unit was 0.7 msec.

The other antidromically activated unit was activated by a stimulation electrode located in the ipsilateral SCN. The threshold current was 1.4 mA (at 0.25 ms). The latency of the response to stimulation at twice the threshold current level was 15.5 ms. A collision of the antidromic response with a spontaneous action potential was observed when the stimulation followed the spontaneous action potential by 16 ms or
In response to visual stimulation, both units had "sustained on" responses. A light intensity-response curve was calculated for one of the antidromically activated units. This curve (see Fig. 23a) did not appear to differ from that of other IGL units. One unit did not respond to illumination of the ipsilateral eye, while the other unit was inhibited by ipsilateral eye illumination.
IV.C. Discussion

The results of this study indicate that the GST consists largely of neurons that show a sustained "on" response to light. These cells appear to show monotonic luminance-response functions similar to those of SCN neurons. Many cells respond to photic stimulation of either eye, with inhibition from stimulation of the ipsilateral eye being quite common.

The hypothesis that GST neurons would tonically increase firing rate in darkness (see section IV.A.4.) was not supported by this study. The majority (86%) of units histologically localized to the IGL gave sustained changes of firing rate in response to light stimulation; 87% of these sustained units gave "on" responses. Although this sample of IGL units may include IGL neurons which do not project to the SCN, it is probable that most GST neurons located in the IGL would have sustained "on" responses to light stimulation. This conclusion is supported by the finding that the two IGL units, which were antidromically activated from stimulation in the SCN gave sustained "on" responses to light stimulation. In this respect, these results are similar to those of Groos and Rusak (1984) who found that in the rat the majority of LGN neurons antidromically activated from SCN stimulation had
sustained "on" responses to light.

There are several possible explanations for the failure to support the initial hypothesis. First, the similarity of the pattern of phase shifts caused by neuropeptide Y injections to that seen after dark pulses may be purely coincidental. The mechanism by which injection of neuropeptide Y causes a phase shift may be different from and independent of the mechanism by which a dark pulse causes a phase shift. Second, the injections of neuropeptide Y in the study of Albers and Ferris (1984) probably involved non-physiological amounts of the peptide. Certainly, the available pool of peptide in extracellular space was not as specific in location as it is under physiological situations. An input to a system given in non-physiological amounts probably results in disruption of normal activity of the system and may result in an inhibitory effect, even if this input typically has an excitatory effect when applied in physiological amounts (see Isaacson, 1981). This same critique may be applied to the study of phase shifts after electrical stimulation of the geniculate (Meijer et al., 1984). Again, a volley of artificially induced electrical activity may disrupt the normal function of the GST and cause phase shifts unrelated to the normal effect of physiological activation of the GST. Both of these studies may tell
us more about the behavioral effects of disruption or suppression of GST neurons than about effects of GST activation. An approach involving a more physiological level of activation of the GST may indicate that activity of these neurons produces behavior typically associated with the perception of light, not darkness. This result would correspond well with extracellular recordings of these cells' responses and also with some of the behavioral results of GST ablation (e.g., the dramatic changes in wheel-running rhythms seen under constant light in GST-ablated hamsters; see Chapter II.).

Visual responses of units in the anterior vLGNei appeared more similar to those in the remainder of the vLGNei than to those in the IGL (see Table 8). Although there are behavioral and anatomical reasons to link the IGL and the anterior vLGNei as a functional unit (see section III.D.), it appears that by this rough physiological assessment, these two areas are not similar. Information on the responses of units identified as GST neurons is especially important in the anterior vLGNei, since the histological evidence suggests that GST neurons make up a minority of the neurons in this area, while they appear much more common in the IGL (see section III.C., figures 16 - 18). Thus, the sample of IGL units recorded in this study may be
assumed to include a high percentage of GST neurons, while the same may not be assumed for the sample of anterior vLGNei units.

Many units in the IGL responded to light stimulation of either eye. Units in the anterior vLGNei were similar to IGL units in that many of them also had binocularly driven visual responses (see Table 9). Binocular responses were rare in other parts of the vLGN. Anatomical descriptions of the retinal input to these areas (see section III.C.) did not suggest this finding, but implied that binocularly driven responses should be common only in the IGL. The percentage of binocular responses observed for all vLGN units in the hamster is more similar to that found in the cat than in the rat or rabbit (see section IV.A.1.).

Responses of binocularly-driven IGL and vLGN units to illumination of the ipsilateral eye frequently involved an inhibition of firing rate at light onset (see Table 10). In many cases, these responses were opposite to the responses to illumination of the contralateral eye. It is possible that this type of response to illumination of the ipsilateral eye was even more common than is suggested by the data, since many units in the IGL and vLGN had extremely low maintained firing rates in darkness. In these cases it would be difficult to observe the effects of an inhibitory input.
if it were presented alone.

The functional role of such an inhibitory input is unclear, but it may act to decrease the response of the unit to brief changes in light intensity. In most cases, the inhibition did not last longer than 30 s. Since a binocular input is probably the natural stimulus for these units, this ipsilaterally driven inhibition would make the unit more sluggish to natural illumination than is observed in the responses to contralateral eye illumination. It is likely that the circadian system requires input from sluggish cells signalling tonic background luminance rather than from briskly responding cells coding transient changes in luminance.

The inhibitory input seen after illumination of the ipsilateral eye may arise from the direct projection of retinal ganglion cells in the ipsilateral eye to the IGL, or it may be due to input to the IGL from neurons in the contralateral IGL. In the former case, the responses of IGL neurons in the absence of such an inhibitory input are seen when only the contralateral eye is illuminated. In the latter case, however, a cut of the postérieur commissure would be required to completely eliminate the inhibitory input. It would be interesting to examine visual responses of IGL neurons after such a cut. There is evidence from other neural
systems that elimination of an inhibitory commissural connection can alter the time course and the polarity of responses of neurons that normally receive such an input (Shimazu and Precht, 1966; see also, Galiaha and Outerbridge, 1984).

The responses of IGL neurons to varied light intensities appear to follow a monotonic function, with a half-maximal response at approximately $35 \mu W/cm^2$ and saturation at approximately $300 \mu W/cm^2$ (see Fig. 23). The responses of IGL neurons do not appear to differ from those of vLGN units similarly assessed (see Fig. 24) nor from those of SCN units in the hamster (Meijer et al., 1986). Thus, the hypothesis that the GST acts to change the sensitivity of SCN units to light (see section IV.A.4) is unlikely, since in that case one might expect the luminance-response functions of GST neurons to show a different threshold than those for SCN neurons. It is still possible that in intact animals GST input dominates over retino-hypothalamic tract input in the SCN; thus, luminance-response functions of SCN units in GST-ablated hamsters might differ from those of SCN units in intact hamsters.

The luminance-coding properties of IGL and SCN neurons suggest that these units may receive input from tonic, on-center W-cells in the retina (Stone and Fukuda, 1974) that have been termed "luminance units"
These retinal units were reported to show monotonic increases in firing rate when luminance levels were increased. The maintained discharge of these units had a very regular pattern, similar to that of many of the IGL units in the sample in this study. Tonic off-center \( \mathcal{W} \)-cells, on the other hand, did not show a monotonic relationship between maintained firing rate and luminance level in the cat's retina (Stone and Fukuda, 1974). Most other retinal units do not show consistent changes in firing rate when luminance levels are changed (Barlow and Levick, 1969; Stone and Fukuda, 1974).

The response of an IGL unit which was antidromically activated from the SCN has been re-plotted in Figure 27 as a function of the light intensity measured in lux. This allows comparison with measures of natural light levels from Thorington (1985). The working range of this unit, typical of other IGL units (see Fig. 23), allows the coding of light intensities over the brighter half of the twilight period, corresponding to a solar altitude of approximately 0 degrees to -10 degrees. Changes in light intensity during the day would probably not be coded by this unit, at least not in terms of the sustained firing rate of the unit during light exposure.

For some units, however, it was noted that the dark
discharge rate after presentation of a saturating light intensity only very gradually decreased to baseline levels. This observation indicates that these units might code daylight levels of luminance by a different mechanism (e.g., duration of discharge above baseline levels after the light exposure) than the mechanism that they appear to use to code twilight levels of luminance (e.g., maintained firing rate during the light exposure). It is also possible that measures of the luminance-response curves of GST units under more natural illumination conditions (e.g., binocular light presentations, non-atropinized pupils) would differ from the curve analyzed here.

The maximum luminance from a full moon is approximately -0.4 log lux, while that from a moon in the first or third quarters (an apparent half moon) is about -1.4 log lux (Thorington, 1985). These values are at the threshold levels of the luminance-response curve of the unit shown in figure 27. Therefore, it is possible that IGL units would show slight variations in their discharge rate depending on the phase of the moon, but it is unlikely that moonlight is the optimal stimulus for these neurons.

In summary, this study has demonstrated that (1) most visual responses of IGL units are sustained "on" type, (2) visual responses of two confirmed GST neurons
in the IGL were sustained "on" type; (3) many units in
the IGL have binocularly driven visual responses with an
inhibitory ipsilateral input, and (4) the anterior
vLGNéi appears similar to the IGL in the observed
percentage of binocularly driven responses, but differs
from the IGL in the proportion of sustained "on" type
visual responses.
Figure 20. Localization of recording tracts in the hamster brain. A frontal section through the geniculate area of a brain stained with the Kluver-Barrera method is shown. Arrows point to the lesions used to mark the electrode track. Abbreviations: DLGN = dorsal lateral geniculate nucleus, IGL = intergeniculate leaflet. Magnification = 215 X.
Figure 21. The response of a unit in the intergeniculate leaflet of a hamster to 30 minutes of illumination of the contralateral eye, followed by 30 minutes of darkness. The trace at the top of the histogram indicates when the lights were on by a movement upwards.
Figure 22. An example of an IGL unit with a binocular response. The histograms show the response to illumination of (A.) the contralateral eye, (B.) the ipsilateral eye and (C.) both eyes. Light was presented for 10 s after a 20 s period of darkness. The line above each graph indicates the time of light presentation by an upward deflection.
Figure 23. Luminance-response curves of four IGL units. Irradiance of the light source was measured in $\mu$W/cm². Light was presented for at least 60 s to the contralateral eye. Maintained firing rate in the light was standardized by subtracting from it the maintained firing rate in the dark. The mean standard deviation are shown if more than one light presentation was given at that light level. The lines connecting the points are simply to aid visualization of the response, except in (B.) where they indicate the order of light presentations.
Figure 24: Luminance-response curves of two units in the anterior vLGNei. See Figure 23 for further explanation.
Figure 25: Luminance-response curves for two units located in the dLGN. For further explanation of curve A., see Figure 23. Curve B. is from a unit for which the light levels were gradually stepped down and then up. At least 2 min was allowed at each light level and the latter half of this period was used to calculate a mean firing rate.
Figure 26. An example of a successful collision test. The response of a unit in the IGL to electrical stimulation of the SCN was recorded. Stimulations were given at varied delays after a spontaneous action potential. The stimulation response was reliable when stimulation followed a spontaneous action potential at delays of 4, 5 or 6 ms, but only occasionally occurred at a delay of 3 ms. When stimulation was applied 2 ms after an action potential, no response was observed.
Figure 27. The luminance-response function of an IGL unit antidromically activated from the SCN in relation to luminance levels of natural light. The luminance of the light stimulus (presented for at least 60 s to the contralateral eye) is measured in lux. The firing rate was standardized for levels of dark discharge and normalized to a scale from 0 to 1. Levels of natural light (from Thorington, 1985) are indicated above the graph, with the common divisions of twilight indicated (astronomical (A.), nautical and civil).
Table 7. Summary of results from several studies surveying the visual receptive field characteristics of vLGN neurons. "N" refers to the total number of units sampled. All other numbers are percentages of the total sample, displaying the relevant response.

"SUST" indicates a sustained "on" response. "LUM" refers to changes in firing rate that appear to code luminance levels. This group of units are always a subset of the group with sustained "on" responses. "PHAS" refers to a phasic response to visual stimulation. "CONC" or "UNIF" means showing a concentric or uniform receptive field organization. "BIN" indicates a binocular response, while "IPSI" refers to responding only to the ipsilateral eye. "MOV" indicates movement sensitivity. "NR" indicates units with no clear visual response.

Table 7

Percentages

<table>
<thead>
<tr>
<th>Ref</th>
<th>N</th>
<th>SUST</th>
<th>LUM</th>
<th>PHAS</th>
<th>CONC</th>
<th>UNIF</th>
<th>BIN</th>
<th>ITST</th>
<th>MOV</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>52</td>
<td>63</td>
<td>27</td>
<td>23</td>
<td>36</td>
<td>27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>R2</td>
<td>56</td>
<td>89</td>
<td>89</td>
<td>9</td>
<td>8</td>
<td>92</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>R3</td>
<td>122</td>
<td>81</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>109</td>
<td></td>
<td></td>
<td>39</td>
<td>14</td>
<td></td>
<td>5</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>154</td>
<td>14</td>
<td>8</td>
<td>40</td>
<td>19</td>
<td>27</td>
<td>30</td>
<td>13</td>
<td>4</td>
<td>39</td>
</tr>
<tr>
<td>C2</td>
<td>177</td>
<td></td>
<td>15</td>
<td>36</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>293</td>
<td>&lt;11</td>
<td>10</td>
<td>52</td>
<td>20</td>
<td>0</td>
<td>18</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>206</td>
<td>7</td>
<td>29</td>
<td>5</td>
<td>45</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 8. Visual response types of units in the intergeniculate leaflet (IGL) and the ventral lateral geniculate nucleus (vLGN). The number of units classified into each response type is shown. Units in the vLGN are further divided by which subdivision of the vLGN they were located in. "Sust" indicates a sustained response. Data are from 41 hamsters.
### Table 8

<table>
<thead>
<tr>
<th>Area</th>
<th>Sust</th>
<th>Long*</th>
<th>Sust</th>
<th>On</th>
<th>Off</th>
<th>On/off</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGL</td>
<td>50</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td>vLGN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ant-ei</td>
<td>17</td>
<td>1</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>9</td>
<td>8</td>
<td>58</td>
</tr>
<tr>
<td>ei</td>
<td>29</td>
<td>6</td>
<td>3</td>
<td>20</td>
<td>2</td>
<td>14</td>
<td>12</td>
<td>86</td>
</tr>
<tr>
<td>ee</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>iv</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>15</td>
<td>11</td>
<td>33</td>
</tr>
<tr>
<td>All vLGN</td>
<td>56</td>
<td>7</td>
<td>10</td>
<td>42</td>
<td>7</td>
<td>42</td>
<td>33</td>
<td>197</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>12</td>
<td>18</td>
<td>46</td>
<td>8</td>
<td>43</td>
<td>37</td>
<td>270</td>
</tr>
</tbody>
</table>
TABLE 9. Binocular responses of units in the intergeniculate leaflet (IGL) and the various subdivisions of the ventral lateral geniculate nucleus (vLGN).

<table>
<thead>
<tr>
<th>Area</th>
<th>Number</th>
<th>Binocular</th>
<th>Number</th>
<th>Tested</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGL</td>
<td>11</td>
<td></td>
<td>16</td>
<td></td>
<td>69%</td>
</tr>
<tr>
<td>VLGN</td>
<td>Ant-ei</td>
<td>7</td>
<td>12</td>
<td>58%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>/ei</td>
<td>3</td>
<td>17</td>
<td>18%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>.ee</td>
<td>0</td>
<td>5</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>iv</td>
<td>0</td>
<td>2</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>All VLGN</td>
<td>10</td>
<td></td>
<td>36</td>
<td></td>
<td>28%</td>
</tr>
</tbody>
</table>
TABLE 10. Responses of intergeniculate leaflet (IGL) and vLGNei units to ipsilateral (ipsi.) eye illumination. Only those units that responded to contralateral (contra.) eye illumination with sustained responses are included. Abbreviations: F.R., firing rate; Sust., sustained; vLGNei, internal part of the external lamina of the ventral lateral geniculate nucleus.

<table>
<thead>
<tr>
<th>Response to Contra. Eye</th>
<th>Response to Ipsi. Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase F.R.</td>
</tr>
<tr>
<td>Sust. on</td>
<td></td>
</tr>
<tr>
<td>IGL</td>
<td>2</td>
</tr>
<tr>
<td>VLGN</td>
<td>1</td>
</tr>
<tr>
<td>Sust. off</td>
<td></td>
</tr>
<tr>
<td>IGL</td>
<td>0</td>
</tr>
<tr>
<td>VLGN</td>
<td>0</td>
</tr>
</tbody>
</table>
CHAPTER V. General discussion

The experiments described in this thesis were all designed to provide information about the function of the geniculo-suprachiasmatic tract. The phase shifting behavior of hamsters after ablation of the GST was described in chapter II. Hamsters with geniculate lesions that destroyed the GST showed decreased phase-advance shifts to light pulses and dark pulses. Such hamsters did not respond to housing under constant illumination in the same way as did the control hamsters; they were less likely to show "split" rhythms and they expressed rhythms with periods different from those of controls. The experiments described in chapter III characterized the GST anatomically. GST neurons, which were shown to be immunoreactive for neuropeptide Y, were located in the intergeniculate leaflet and the anterior vLGNei. The distribution of retinal afferents in the geniculate area was described in detail. In chapter IV, I described the visual responses of putative GST neurons recorded extracellularly in the IGL and vLGN. Most GST neurons responded to diffuse illumination with a sustained "on" response. Firing rates of these sustained "on" units increased
monotonically with increases in light levels over a working range of about 3 log units. Many putative GST neurons have binocularly driven responses, with the input from the ipsilateral eye being inhibitory.

Since GST ablation altered phase shifting of hamsters exposed to light pulses, it is possible that the GST may play an important role in entrainment (see Pittendrigh and Daan, 1978b); however, this role may be redundant with that served by the direct retinal input to the SCN. Thus, in animals with GST ablation, the direct retinal input to the SCN may be sufficient for normal entrainment. A technique for selectively destroying the retinohypothalamic tract which provides this direct input would be very useful in investigating whether the GST is by itself also sufficient for normal entrainment. To accomplish this surgically is extremely difficult in the hamster, although it might be possible in an animal such as the rabbit in which the SCN is not embedded in the optic chiasm. Alternatively, it might be possible to selectively destroy or inactivate the retinohypothalamic tract using chemical techniques. If the neurotransmitter of the retinohypothalamic tract were known, it might be possible to develop a technique for selectively blocking that neurotransmitter in the SCN.

Determining the function of the GST might be easier
if it were known what type of stimulus normally activates these neurons and, moreover, what effect their activation has on SCN neurons. The results presented in chapter IV indicate that in the hamster most of the GST neurons respond to whole eye illumination with a sustained "on" response and show luminance-coding properties. A similar result was found in the rat (Groos and Rosak, 1982); thus, it is likely that illumination activates most GST neurons. We have very little information from mammals on the question of what effect GST activity has on SCN neurons. Card and Moore (1985) reported that synapses of neuropeptide Y-immunoreactive terminals upon SCN cell dendrites in the rat usually have an asymmetric structure. This type of synaptic structure has often been correlated with excitatory synapses; however, this correlation is too weak to allow any definitive conclusion regarding the effect of these particular synapses. More conclusive evidence would arise from experiments involving electrophysiological recordings of responses of SCN neurons to neuropeptide Y application.

Some results from studies of the Bulla and the Aplysia eye may be relevant to this question. In hamsters, the action of neuropeptide Y, a putative neurotransmitter of the GST, is to produce phase shifts similar to those produced by dark pulses. Jacklet
(1985) has reported that perfusion of the Bulla and Aplysia eye with FMRF-amide, a peptide structurally related to neuropeptide Y, suppressed compound action potentials, and preliminary results indicated that FMRF-amide may shift the circadian rhythm of compound action potentials in a manner similar to dark pulses. These results indicate that oscillators in the Bulla and Aplysia eye may resemble the mammalian circadian system in their response to this type of peptide. In the Bulla eye, dark pulse-like phase shifts can also be produced by injection of hyperpolarizing current into the putative pacemaker neurons (the basal retinal neurons), while injections of depolarizing current into these neurons produces light pulse-like phase shifts (McMahon and Block, 1985). These results indicate that in the pacemaker of the Bulla eye an inhibitory input produces effects similar to dark pulses. It would be interesting to know if FMRFamide causes hyperpolarization of the basal retinal neurons of the Bulla eye. It is tempting to draw an analogy between the effects of FMRFamide on the Bulla eye with the effects of neuropeptide Y on the mammalian suprachiasmatic nucleus, but it is obvious that more information is needed on both systems in order to assess the significance of these parallel features. We especially need more information on the physiological effects of activation of the GST on SCN neurons.
From the above discussion, it appears likely that (1) illumination of the eyes causes increased firing of GST neurons, and (more tentatively) (2) that GST neurons have an inhibitory effect on SCN neurons. Given these assumptions, it is possible to form an hypothesis explaining the behavioral effects attributable to loss of GST input (see Chapter II). Under one theoretical framework (Enright, 1980), the loss of an inhibitory input to the pacemaker during illumination would be interpreted as having the effect of lowering the threshold of the system.

Enright's proposed model of a circadian pacemaker consists of many separately oscillating components ("pacers") which are mutually entrained. Critical to his model is the stipulation that the entraining feedback from pacer discharge to the pacers is nonlinear. He formulates this feedback as arising from a separate, non-oscillatory "discriminator" component (see Figure 28A). When summed pacer discharge is greater than a critical level (the threshold of the system), then the discriminator provides excitatory feedback to the population of pacers, accelerating the cycles of all nondischarging pacers. Some pacers with periods much longer than the average period of the system will never contribute to the achievement of the threshold level of summed pacer discharge; these are
referred to as the unentrained pacers. It is not necessary for a general model such as Enright's that the discriminator be a separate component, anatomically distinct from the pacers. The function of the discriminator could arise from direct interactions among the pacers; however, it is crucial that such interaction be nonlinear and include a threshold level.

Enright's single pacemaker model as diagrammed in Figure 28A cannot easily account for the occurrence of split rhythms. To account for this phenomenon, Enright proposed that the model be elaborated to include two pacemaker systems which are normally coupled together. A possible form that this coupling might take would be that of a weak inhibitory interaction such that when one discriminator is active it tends to slightly raise the threshold of the other discriminator. Computer simulations of such a system indicate that when the threshold is low the activity of the two discriminators remains in phase. When threshold is raised, however, the two discriminators gradually move out of phase with each other, until they reach the point where there is no overlap in the time of activity of the two discriminators, 120 to 180 degrees out of phase. They would then lock at this phase relation and run with similar periods until the threshold is again lowered, at which point they would gradually return to the initial

Enright hypothesizes that light increases the threshold in nocturnal rodents; therefore, a GST-ablated hamster would be predicted to show relatively less of an increase in threshold under illumination. Predictions of the behavior of a nocturnal rodent's pacemaker system after such a relative decrease in the threshold level include (1) a shorter period of the rhythm under continuous illumination, (2) decreased phase advances to light pulses, and (3) possibly a decreased occurrence of "splitting" of the rhythm into two components (see Enright, 1980). Figure 28B diagrams how a relative decrease in threshold could lead to a decrease in the period of the rhythm under constant illumination. The system would reach a lower threshold level (e.g., 20% of the pacers discharging) more quickly than a higher threshold (e.g., 40% of the pacers discharging). Thus, the output of the system would show a shorter period under a lower threshold; this result was observed in computer simulations of the model (Enright, 1980, p. 71). The second predicted effect, that of decreased phase advances to light, is due to the magnitude of the phase advance of this type of system being dependent upon the magnitude of the light-induced change in threshold. The third effect, that of decreased occurrence of splitting, could be predicted by the
hypothesis that splitting occurs only when the threshold is high; thus, light may never increase the threshold of GST-ablated animals to the critical level needed to induce splitting. These three predicted effects mirror quite closely the observed effects of GST-ablation described in chapter II.

It is also possible to derive further predictions from Enright's model if GST ablation is assumed to cause a relative decrease in the threshold during illumination. One such prediction is that phase delays to light pulses should be decreased after GST ablation (due to the same reasoning which led to the prediction of a decrease in advancing phase shifts). This prediction is not substantiated by the data presented in this thesis; phase delays were not changed after GST ablation. In fact, there is one report of increased phase-delay shifts to light pulses after GST ablation (Pickard and Ralph, 1984). The failure of this prediction indicates that the application of this model to these data requires additional assumptions. Enright's model can account for asymmetric effects on the advance and delay portions of a phase response curve if a change in the number of unentrained long-period pacers is invoked (Enright, 1980, pp. 146-147). Such an elaboration of the hypothesis would not have a large effect on other aspects of rhythmic behavior, since
these unentrained long-period pacers normally provide only a small contribution to the output of the system.

My original hypothesis leads to several further predictions, derived from results of computer simulations described by Enright; these predictions include (1) changes in the precision of activity rhythms under LL, (2) an increase in the intensity or amount of activity in LL, and (3) more positive phase angles of entrainment after GST- ablation (Enright, 1980, pp. 70, 71 and 123). Data from hamsters with primary optic tract lesions (Rusak and Boulos, 1981) may provide support for these predictions; however, in view of the complications inherent in interpreting effects of such lesions (see section II.A.2.), hamsters with lesions similar to the ones used in the present study should be used to more definitively test these predictions. These predictions should be tested in further experiments since they provide critical tests of the hypothesis that GST ablation has effects similar to a relative decrease in the threshold in Enright's model.
Figure 28. (A) Schematic representation of a possible structure which might mediate the kinds of interactions between pacers (P1, P2,...PN) and a discriminator (D) described in Enright's model (from Enright, 1980).

(B) Schematic representation of the manner in which a decrease in the threshold level could lead to a decrease in period. The first graph represents the behavior of the system at one threshold level (T1). The second graph shows how the frequency of the system is increased when threshold is decreased (T2).
APPENDIX A. Afferent and efferent connections of the vLGN and IGL

This section will discuss in detail what is known about the afferent and efferent connections of the vLGN and IGL. Afferents and efferents are discussed together because many of the connections of the vLGN are reciprocal. In many cases, the studies discussed below did not clearly distinguish between the IGL and the vLGN. I have imposed this distinction where possible, based on descriptions or figures. In most cases, however, I have discussed the work as the authors presented it, thus including in the term "vLGN" the structure I call the IGL.

In general, the vLGN receives visual input both directly from the retina and indirectly from the visual cortex. The vLGN has connections with several subcortical visual centres, such as the lateral terminal nucleus, the medial terminal nucleus, the superior colliculus, the pretectum and the contralateral vLGN. The zona incerta and many brainstem nuclei have reciprocal connections with the vLGN. Finally, the vLGN sends a non-reciprocal projection to the pons and the SCN. The projection to the SCN is discussed in section III.A.4.
A.1. Cortical afferents to the vLGN

The vLGN receives a projection from the visual cortex. Several studies have attempted to determine the location of the cells of origin of this projection.

Sefton, Makay-Sim, Baur and Cottee (1981) studied in detail the laminae and areas of origin of the descending visual cortical projections via small horseradish peroxidase (HRP) injections into the vLGN of rats. The pattern of labeling was compared with that seen after similar injections placed into surrounding or related areas. Injections which included the vLGN labeled pyramidal cells in the ipsilateral cortical lamina V. The diameters of these cells averaged 13.8 ± 1.3 μm (n = 130). In all three cortical areas (17, 18 and 18a), the caudal two-thirds of the cortical area contained the densest population of labeled cells after vLGN injections. Smaller pyramidal cells in lamina VI were always labeled in these animals, particularly in area 17.

After dLGN and reticular thalamic nucleus injections, labeled cells were observed only in lamina VI of areas 17, 18 and 18a. Since all vLGN injections included the dLGN, the labeled cells observed in lamina VI after vLGN/dLGN injections might be wholly attributable to retrograde transport from neurons other
than those in the vLGN.

After superior colliculus injections, labeled neurons were observed in lamina V. Since some cortico-collicular fibers pass close to, and through, the LGN, it is possible that after injections of the LGN labeled neurons in lamina V could be due to uptake from damaged cortico-collicular fibers. However, since a large lesion destroying the rostral superior colliculus (where the majority of collicular afferents are thought to enter from the retina and the cortex) did not prevent HRP injections into the vLGN from labeling lamina V neurons, this interpretation is not likely. The sizes of cortical labeled cells after vLGN or superior colliculus injections are similar, and thus it remains possible that the axons of some cells in lamina V might branch to supply both nuclei. Reports by Cosenza and Moore (1984) and Schober and Gruschka (1983) basically confirm the conclusions of Sefton et al. (1981).

In the cat, the projection from the cortex to the vLGN appears similar, although arising from a greater number of cortical areas. Hughes and Chi (1981) studied this projection by injections of HRP into the dLGN and vLGN of cats. Labeled cortical cells identified as projecting to the vLGN were in layer V of areas 17, 18, 19, 20, 21 and in the antero-medial lateral suprasylvian area. Of these, area 20 had the most cell labeling with
parts of area 17 also showing dense labeling.

Species differ in the exact distribution of cortical afferents within the vLGN. In the rat, area 17 projects to the external division of the vLGN (Takahashi, 1985), sparing the most anterior part of this division (Schober and Gruschka, 1983). Area 18 projects to the internal division, while area 18a projects to both the internal and external divisions of the vLGN (Takahashi, 1985). All of these projections are solely ipsilateral. The IGL in rats does not appear to receive cortical input (Schober and Gruschka, 1983; Swanson et al., 1974; Takahashi, 1985).

In hamsters, small injections of tritiated amino acids into area 17 produces patches of label in the external division of the ipsilateral vLGN (So and Jen, 1982) and possibly extending into the internal division and the zona incerta (Lent, 1982). There appears to be some topographic organization, with the upper retinal representation in area 17 projecting to the ventro-caudal vLGN, while the lower retinal representation (more caudal area 17) projects to the dorso-rostral quadrant (Lent, 1982).

A columnar organization is observed in the cortical projection to the vLGN of rabbits. The projection of visual cortical area 1 (striate and peristriate cortex) to the vLGN in albino rabbits was studied by Giolli et
al. (1978) using cortical injections of tritiated leucine. In all cases, label was seen in a dense strip or column in the vLGN. Localization of the strip in the vLGN appeared to vary systematically with injection site location in the visual cortex. A study done by Hollander, Tietze and Distel (1979) was similar to that of Giolli et al. (1978) except that pigmented rabbits were used. These rabbits received small injections of tritiated leucine into the striate cortex. Injections were all placed lateral to the lateral sulcus and about halfway between the rostral border of the striate cortex and the caudal edge of the hemisphere. Labeling in the vLGN appeared in horizontal and transverse sections as an oval spot interrupted by small gaps, resulting in a reticular pattern.

Following up on several earlier studies (Graybiel, 1974; Kawamura, Sprague and Niimi, 1974), Updyke (1979) used autoradiography to more precisely map the cortical projections to the vLGN in the cat. Only very posterior injections into area 17 produced label in the vLGN. Labeling was restricted to the ventrolateral parts of the external division (Jordan and Hollander's vLGNc and vLGNe; see section III.A.1.). There was no labeling in the dorsal and caudal extremes of the vLGN. Injections in all parts of area 19 also produced labeling in the same divisions of the vLGN (vLGNc and vLGNe) and also in
the rostral stalk (vLGNb). Injections into area 18 produced labeling similar to that seen after area 19 injections except that labeling was less dense. An orderly distribution of label in the vLGN seen after small cortical injections suggested some topographic organization, although the lack of a vLGN projection from rostral parts of area 17 is puzzling.

In general, cortical afferents to the vLGN appear to arise from lamina V of visual cortical areas. The external division of the vLGN is the most common recipient of these afferents, although species differ in the exact distribution of cortical terminals. There is some evidence for a topographic organization of this projection in many species (i.e., hamster, rabbit and cat).

A.2. Connections of the vLGN with subcortical visual centres

The vLGN has connections with several subcortical visual centres. vLGN neurons project to the lateral terminal nucleus, the medial terminal nucleus, and have reciprocal connections with the superior colliculus, the pretectum and the contralateral vLGN (see Tables 2 and 3).

An efferent projection from the vLGN to both the ipsi- and the contralateral lateral terminal nucleus has
been described in both the rat and the cat (Legg, 1979; Ribak et al., 1975; Swanson et al., 1974). Mackay-Sim et al. (1983) but no others (see Table 2) reported that HRP injections in the vLGN of rats labeled neurons in the contralateral lateral terminal nucleus "in a clearly defined line just caudal and medial to vLGN and ventral to the medial geniculate nucleus". From the description of the location of these neurons, it appears likely that they were actually located in the caudal IGL and not in the lateral terminal nucleus.

A projection from the vLGN to the ipsilateral medial terminal nucleus has been described in the cat (Edwards et al., 1974; Graybiel, 1974) and in one study using rats (Graybiel, 1974).

The vLGN has a reciprocal connection with the superior colliculus. The projection of the vLGN to the superior colliculus appears to be entirely ipsilateral (but see Matute and Streit, 1985). Descriptions of where in the superior colliculus afferents from the vLGN terminate vary widely.

In the rat, Swanson et al. (1974) described silver grains over all layers of the superior colliculus except the stratum zonale. Label was especially dense in the lateral half of the stratum griseum intermediale. Ribak and Peters (1975) reported that the superior colliculus had the densest labeling in the stratum opticum,
especially in the medial third. All other parts of the superior colliculus had labeling greater than background in this study. Graybiel (1974) described label in the superior colliculus distributed mainly in the stratum opticum and statum intermediale, while lighter labeling was seen in the stratum griseum superficiale. Legg (1979) allowed longer survival times after anterograde tracer injections, hoping for a clearer picture of efferent projections, and found that label was concentrated in the rat's superior colliculus in two layers, one on the boundary between stratum opticum and stratum griseum superficiale and one between stratum opticum and stratum griseum intermediale.

In the cat, Swanson et al. (1974) reported that the grain densities in the ipsilateral superior colliculus, although higher than background, were not as high as in the rat brain. Silver grains were seen over all layers of the superior colliculus, including the stratum zonale. Edwards et al. (1974), allowing longer survival times, describe the projection from the vLGN to the superior colliculus in the cat as terminating in the stratum opticum, the stratum griseum intermediale and the deeper portions of the stratum griseum superficiale.

Matute and Streit (1985) concluded from superior colliculus HRP injections that the vLGN probably provides the greatest thalamic source of afferents to
the superior colliculus. They also reported that no vLGN neurons were labeled following injections of tritiated aspartate or γ-aminobutyric acid (GABA) into the superior colliculus of rats or rabbits. This indicates that the vLGN afferents to the superior colliculus probably do not contain glutamate, aspartate or GABA.

The location of the vLGN neurons afferent to the superior colliculus has been described in the cat (Edwards et al., 1979; Kawamura et al., 1978) and the rat (Brauer and Schober, 1982). In the rat, neurons labeled after superior colliculus HRP injections were found only in the external layer of the vLGN. These neurons were similar to class 1 neurons seen in Golgi material (Brauer and Schober, 1982; see Table 1).

The superior colliculus does not just receive a projection from the vLGN; neurons in the superior colliculus also project to the vLGN (Cosenza and Moore, 1984; Graybiel, 1974; Pasquier and Villar, 1982a; Takahashi, 1985). In the rat, this projection appears to arise from neurons in the stratum griseum superficiale and, to a lesser degree, the stratum intermediate of the superior colliculus (Cosenza and Moore, 1984; Pasquier and Villar, 1982a). The projection from superior colliculus neurons terminates in the external division of the vLGN in rats (Takahashi,
The vLGN has reciprocal connections with the pretectum. The projection from the vLGN to the pretectum in the rat originates from class 1 neurons (see Table 1) in the dorsolateral vLGN and perhaps in the IGL (Brauer et al., 1984). Reports of the location of these neurons in the cat vLGN differ (Berman, 1977; Kawamura et al., 1978). However, it is interesting that, as in the rat, Kawamura et al. (1978) report that vLGN neurons projecting to the superior colliculus and the pretectum in the cat have similar morphology and location. There is electrophysiological evidence (Hada et al., 1986) indicating that some vLGN neurons in the cat project to both the superior colliculus and the pretectum.

The terminal field of this projection from the vLGN to the pretectum varies slightly between the rat and the cat. In the rat, vLGN afferents terminate in the ipsilateral and contralateral olivary pretectal nuclei, in the contralateral nucleus of the optic tract (Swanson et al., 1974) and in the ipsilateral anterior pretectal nucleus (Legg, 1979). In the cat, Swanson et al. (1974) report that both the ipsi- and the contralateral nuclei of the optic tract were labeled by injections of tritiated amino acids into the vLGN. The ipsilateral anterior and medial pretectal nuclei were lightly
labeled, while on the contralateral side, grains were observed only in the medial pretectal nuclei. On the other hand, Edwards et al. (1974) reported that after injecting anterograde tracers into the vLGN of the cat the ipsilateral olivary pretectal nucleus, the nucleus of the optic tract and the posterior pretectal nucleus were densely labeled. Lighter labeling was observed throughout most of the rostrocaudal extent of the pars compacta of the anterior pretectal nucleus and in the suboptic pretectal nucleus (situated between the nucleus of the optic tract and the posterior pretectal nucleus). On the contralateral side, fibers terminated only in the nucleus of the optic tract.

The projection from the pretectum to the vLGN originates from neurons in the ipsilateral anterior pretectal nucleus and bilaterally in the olivary and posterior pretectal nuclei and the nucleus of the optic tract in rats (Cosenza and Moore, 1984; Mackay-Sim et al., 1983).

Although there is no information on what part of the vLGN in the rat receives pretectal input, Berman (1977) thoroughly studied the efferents of the cat pretectum using injections of a tritiated proline and leucine solution. After large injections involving most of the pretectal complex and encroaching upon nearby areas, fibers terminated in the middle of both the dorsoventral
and mediolateral extents of the vLGN. In some cases, label was observed in the contralateral vLGN after large pretectal injections.

Cases with more localized injections were analyzed to determine the projections of individual pretectal nuclei in the cat. Injections localized to the nucleus of the optic tract led to terminal labeling in the ipsilateral vLGN localized predominantly but not exclusively in the vLGNb, partially overlapping the region of the vLGN receiving retinal afferents. A band of label was seen at rostral levels of the vLGN extending dorsomedially to ventromedially, lying midway between the optic tract and the dLGN. Further caudally the label is heaviest in the ventromedial part of this band.

Labeled terminals from the olivary pretectal nucleus were found in the contralateral vLGN located around the large neurons in the vLGNb. This projection was much less dense than the projection from the nucleus of the optic tract to the ipsilateral vLGN. The medial pretectal nucleus was never labeled without involvement of adjacent structures, so it is impossible to determine if this nucleus projects to the vLGN. Injections in the anterior pretectal nucleus, the posterior pretectal nucleus, the nucleus of the posterior commissure or the central tegmental fields did not label terminal fields
in the vLGN.

In summary, it appears that the strongest connections of the pretectum and the vLGN involve the olivary pretectal nucleus and the nucleus of the optic tract. These are the retinorecipient pretectal nuclei (Berman, 1977). The pretectum has long been known to lie in the pathway mediating the pupillary light reflex. Recent evidence indicates that the olivary pretectal nucleus neurons provide signals for pupillary constriction to neurons of the oculomotor nucleus in rats (Trejo and Cicerone, 1984). Thus, it is possible that the connections of the vLGN and the olivary pretectal nucleus may modulate the pupillary light reflex or may provide feedback about pupil diameter to other parts of the visual system. The neurons of the nucleus of the optic tract appear to play a role in controlling the slow phase of optokinetic nystagmus in cats (Ballas and Hoffmann, 1985). It is unclear what role is played by vLGN input to the nucleus of the optic tract, but there are reports that some vLGN neurons in the cat fire in a fixed temporal relation to nystagmic eye movements (Magnin and Putkonen, 1978).

The IGL projects to the contralateral IGL in rats and hamsters (Brauer et al., 1984; Cosenza and Moore, 1984; Graybiel, 1974; Mackay-Sim et al., 1983; Pasquier and Villar, 1982a; Pickard, 1982, 1985; Swanson et al.,...
1974; but see Ribak and Peters, 1975). These neurons as labeled by HRP injections in the contralateral vLGN were described as elongate cells (mean diameter: 7.6 ± 1.8 μm, n = 24; Mackay-Sim et al., 1983). Brauer et al. (1984) note that IGL neurons that project to the contralateral IGL resemble their class 3 neurons (see Table 1).

It is obvious from this review that the vLGN has widespread projections to subcortical visual areas. Swanson et al. (1974) commented that, with few exceptions (most notably, the dLGN) every brain area which receives a direct projection from the retina also receives a projection from the vLGN. It is not known if any of these projections preserve the topographic organization apparent in the projections from the retina and the visual cortex to the vLGN.

A.3. vLGN connections with the zona incerta, pons, and other areas

The vLGN appears to have reciprocal connections with the neighboring zona incerta. Evidence that the zona incerta projects to the vLGN in rats comes from two sources. First, injections of a tritiated leucine-proline mixture into the zona incerta produced labeling in the IGL (Watanabe and Kawana, 1982); second, injections of HRP in the vLGN label large polygonal neurons of the ipsilateral zona incerta (Pasquier and
The reciprocal projection from the vLGN to the zona incerta has been noted in experiments involving injections of anterograde tracers into the vLGN of rats (Brauer et al., 1984; Legg, 1979; Ribak and Peters, 1974; Swanson et al., 1974) and cats (Edwards et al., 1974; Swanson et al., 1974). Injections of retrograde tracers into the zona incerta of rats labeled cells in both the internal and external divisions of the ipsilateral vLGN (Shammah-Lagnado et al., 1985). Thus, this "reciprocal" connection of the vLGN and zona incerta does not actually involve the same parts of the vLGN; the vLGNi and vLGNe neurons project to the zona incerta, while the IGL neurons are the recipients of a zona incerta projection.

Injections of anterograde tracers into the vLGN label a small terminal field along the midline of the rostral pons in rats and cats (Brauer et al., 1984; Edwards et al., 1974; Graybiel, 1974; Legg, 1979; Ribak and Peters, 1975). Cells in this area of the pons project to the cerebellum (Graybiel et al., 1974) indicating a possible route for visual information relayed via the vLGN to reach the cerebellum. Schober (1983) placed HRP into the central pons and found labeled cells, resembling class 4 cells (see Table 1), located in the IGL.
There is an interesting suggestion that a pathway from the vestibular nuclei to the vLGN and then to the thalamic intralaminar nuclei could be involved in integrating vestibular and oculomotor information with early levels of motor processing. Most of the evidence for this comes from work done using cats. Magnin and Kennedy (1979) made small HRP injections in the vLGN of cats where vestibular-related unit activity had been recorded. The injections were found to be centered on the vLGNb and vLGNd. Many labeled neurons in the contralateral inferior and medial vestibular nuclei and scattered neurons in the ipsilateral inferior, medial and superior vestibular nuclei were observed. Similarly, Pasquier and Villar (1982a) reported that HRP injections in the vLGN of the rat labeled neurons in the ipsilateral vestibular nuclei.

The projection from the vLGN to the thalamic intralaminar nuclei has been shown by several techniques, but only in cats. Magnin and Kennedy (1979) injected tritiated amino acids in the vLGN at sites where vestibular-related unit activity was recorded and found label in the paracentral nucleus and the central lateral nucleus of the ipsilateral intralaminar nuclei. They also injected HRP into the intralaminar nuclei after first recording unit activity and finding activity related to saccadic eye movements. Labeled neurons were
found in the rostral third of the vLGN, in the vLGNa and vLGNb divisions, with some in the vLGNd. Similarly, Kawamura et al. (1978) injected HRP into the intralaminar nuclei of cats and found multipolar, medium sized labeled neurons in the medial vLGN (see also, Kaufman and Rosenquist, 1985).

The vLGN has connections with many brainstem nuclei (see Tables 2 and 3). The projection from the locus coeruleus to the vLGN appears to originate from noradrenergic neurons in rats (Jones and Moore, 1977; Kromer and Moore, 1980).

The projection from the cuneate nucleus of the medulla in rats appears to terminate specifically in the IGL (Feldman and Kruger, 1980). The cuneate nucleus is the area of the origin of the fibers of the medial lemniscus.

Although the vLGN receives retinal input and has connections with most of the areas of the brain concerned with processing visual information, it is clear from these other connections that the vLGN is not concerned solely with vision. The connections with the pons and the zona incerta suggest a role in motor systems, while other connections suggest a role in processing vestibular information. Thus, the most likely hypothesis of a function for the vLGN from what is known of the anatomy would be a very broad hypothesis
of a role in visuomotor integration. Although there is no reason to link the IGL, specifically, with the vestibular system, it is clearly connected to the pons and zona incerta, suggesting a possible role for the IGL in visuomotor integration.
APPENDIX B. Electrophysiological studies of the vLGN

The visual response characteristics of vLGN neurons vary rather dramatically across species. Therefore, I will discuss separately the three species that have been used in previous studies (the rat, rabbit and cat). The results are summarized and compared in section IV.A.1. It may be useful to refer to Table 7 while reading the following sections.

B.1. Electrophysiology of the vLGN in rats

The visual and electrical response properties of extracellularly recorded units in the vLGN and dLGN were compared by several authors. This is a convenient comparison, since recording electrodes pass through the dLGN on the way to the vLGN, but it is not a particularly relevant comparison. The dorsal and ventral lateral geniculate nuclei are derived from embryologically distinct tissues (dorsal thalamus vs. ventral thalamus). The major function of the dLGN is to relay visual information to the visual cortex, while the function of the vLGN must be quite different since no vLGN neurons project to cortex. However, since a good deal is known about response characteristics of dLGN neurons, contrasting the responses of vLGN and dLGN
neurons may prove helpful at least in highlighting the different functional roles of these nuclei.

The earliest study contrasting dLGN and vLGN unit responses in rats was reported by Hale and Sèfton (1978). Most of the vLGN units recorded responded to visual stimuli. All visually responsive units had "on" responses to light presentations. The majority of these units showed a sustained "on" response with no "off" response, while a few units gave weak transient "on" responses. No units showed an inhibition of firing rate when the area surrounding, but not including the receptive field was illuminated. Thus, no units showed active suppressive surrounds; however, about one half of the sustained-type units gave a weaker response to a large light spot than to a light spot the size of the receptive field center, indicating the presence of a silent suppressive surround. The rest of the sustained-type units lacked a suppressive surround region; these units showed a greater response to a large light spot than to a center-sized spot. The units without suppressive surrounds all varied their discharge rates as the intensity of ambient illumination was varied.

The majority of dLGN units recorded gave transient responses to light presentation. Unlike vLGN units, many dLGN units gave "off" responses and had active
surrounds. The receptive field center sizes of the dLGN units were smaller than those of vLGN units (mean ± s.d. = 7 ± 3.1 vs. 14 ± 7 degrees, respectively).

Conduction velocities of retinal afferents were measured by taking the difference in latency of response to electrical stimulation of the optic nerve and the optic tract. The range of conduction velocities observed indicated that both the dLGN and vLGN were innervated by retinal fibers from all 3 conduction velocity groups (3, 6, and 13 m/s); but the vLGN appeared to be innervated by a higher proportion of the slowly conducting group.

A second study comparing visual responses of vLGN and dLGN units in rats was reported by Sumitomo et al. (1979). Criteria for identification of vLGN units included orthodromic responses to visual cortex as well as optic tract electrical stimulation. Since presumably not all vLGN units receive visual cortex input, it must be assumed that this is a rather selective sample of vLGN units. In fact, this criterion may lead to selectively not including units in the IGL and the rostral vLGN in the sample (see Schober, 1983).

A total of 56 vLGN units were analyzed for visual receptive field properties. No visually responsive units were found in the vLGNi. Three of the units responded to stimulation of the ipsilateral eye and were
not analyzed further. Of the 53 units that responded solely to the contralateral eye, most units gave sustained "on" responses to stimulation of the receptive fields with a spot of light. Four units showed a suppression of firing rate at the onset of a light stimulus. One unit was phasically suppressed at both the onset and the end of illumination. The one remaining unit appeared to be movement sensitive. Most receptive fields had no surround characteristics. Ten units of the sustained "on" type were tested with light spots of different luminances. All of these units increased their discharge rates as illumination intensity increased. Receptive field center diameters ranged from 6.3 to 45.6 degrees (mean = 22.3 degrees). As noted by Hale and Sefton (1978), on average, receptive fields of vLGp units were larger than those of dLGp units.

The responses to double stimuli applied to the optic tract at intervals varying from 2 to 500 ms were recorded for both dLGp and vLGp units. The degree of postexcitatory inhibition was judged by the firing probabilities to the second stimulus which, when plotted against the intervals between conditioning and test shocks, gave a recovery curve for the unit. Of 33 vLGp units tested, 28 showed a smooth recovery curve. The slowest recovering unit in this group showed complete
recovery by 40 ms. Five of the 33 units showed an oscillatory recovery. The recovery curves of dLGN units showed a long lasting complete inhibition (lasting for 100 to 200 ms), followed by a strong brief late discharge and then a shorter period of complete inhibition occurring at about 300 ms. The strong inhibition of firing in the dLGN after a conditioning shock has been postulated to be due to a recurrent inhibitory pathway involving neurons located in the thalamic reticular nucleus (Sumitomo et al., 1976). These results indicate that such a recurrent inhibitory input is probably not common in the vLGN. A similar result was more briefly reported by Hale and Sefton (1978).

The mean synaptic delay of vLGN units was 0.64 ms, a value comparable to a previously derived value of 0.73 ms for dLGN units (Sumitomo et al., 1969). Thus, vLGN units are assumed to be monosynaptically innervated by optic nerve fibers, as are dLGN units.

Conduction velocities of optic nerve fibers afferent to vLGN units ranged from 2 to 13 m/s (mean = 6 m/s). In contrast, conduction velocities of optic nerve fibers innervating dLGN units showed a different distribution, ranging from 2 to approximately 22 m/s. Thus, retinal afferents to the vLGN do not include cells with more rapid conduction velocities. This observation, combined
with the observation that when velocity of the light stimulus was varied, most vLGN units preferred slow movements, led the authors to suggest that vLGN neurons are innervated by the class of retinal ganglion cells characterized by slow conduction velocities and sluggish responses (W-cells; Stone and Fukuda, 1974).

Hayashi and Nagata (1981) reported on a study of visual responses of vLGN units, identifying with a stimulating electrode those vLGN units projecting to the superior colliculus. They used the criteria of Sumitomo et al. (1979) to identify vLGN units and therefore sampled only those vLGN units responding to visual cortex and optic chiasm stimulation.

A total of 136 vLGN units were studied. Most of the units (125/136) responded to stimulation of the superior colliculus, and fifty units were antidromically activated. Some units which were antidromically activated were also orthodromically activated after superior colliculus stimulation. Units that responded antidromically to superior colliculus stimulation had latencies to optic chiasm stimulation of 1.4 to 4 ms (mean = 2.2 ± 2.7 ms), while those that responded orthodromically had latencies of 1 to 12 ms (mean = 3.4 ± 2 ms). Thus, retinal afferents to vLGN neurons that project to the superior colliculus appear to have somewhat faster conduction velocities than those
afferent to other vLGN neurons.

The visual responses of vLGN units projecting to the superior colliculus were similar to those of the vLGN as a whole. All but one of the 122 vLGN units tested for visual responses were driven only by the contralateral eye. Sustained "on" responses were the most common (81%), while a sizable minority (14%) of the units were movement sensitive.

Histological reconstruction of the recording tracks indicated that vLGN neurons projecting to the superior colliculus are scattered diffusely within the vLGN. It was noted that it was still possible to orthodromically activate units from superior colliculus stimulation if the rat was bilaterally enucleated, indicating that this response to stimulation is probably from direct superior colliculus afferents and not from activation of collaterals of retinotectal afferents.

Nagata and Hayashi (1984), using the same criteria for identification of vLGN units as in the previous study, examined the visuotopic organization of the vLGN. An early study of the visuotopic organization of the vLGN in rats (Montero et al., 1968) indicated that in the vLGN the nasal visual field is represented dorsally, while the temporal field is represented ventrally. The study of Nagata and Hayashi confirmed this conclusion. Furthermore, neurons in the medial
vLGNe were found to have receptive fields in the lower visual field while those in more lateral parts of the vLGNe had receptive fields in the upper visual field.

Units in the rostral vLGN have receptive fields located in the upper temporal visual field. The very rostral vLGN is unique in having a representation of the extreme upper visual field (20 - 60 degrees above the horizontal meridian), an area that is not represented in the rest of the vLGN. The nasal field is not well represented in the rostral vLGN relative to its representation in the remainder of the vLGN.

Very few responses of vLGN units to stimulation of the ipsilateral eye were observed. The 4 neurons that did respond to stimulation of the ipsilateral eye all had sustained "on" responses and receptive fields in the nasal visual field. No binocular facilitation or inhibition was observed.

Groos and Rusak (1982) reported that 23% of vLGN units in the rat showed uniform receptive fields and sustained responses to light. Most (81%) of these units increased their firing rates with increasing light intensity. Of a total sample of 353 vLGN units, 12 units were antidromically activated by electrical stimulation of the suprachiasmatic nucleus. Seven of these units showed sustained "on" responses to light, two units showed sustained "off" responses, one unit
gave phasic responses and two units did not respond to retinal illumination.

In summary, most visually responsive neurons in the vLGN of the rat appear to have sustained "on" responses to light. Two studies (Hayashi and Nagata, 1981; Sumitomo et al., 1979) sampled only vLGN units responding orthodromically to visual cortex stimulation. These two studies found a slightly higher percentage of sustained "on" type responses than the one study (Hale and Sefton, 1979) that sampled vLGN units less discriminatively (89% and 81% vs. 73% of visually responsive units were sustained "on" type). Sizes of receptive fields of vLGN units are large relative to those of dLGN units. No vLGN units with active surround regions to their receptive fields have been described, although some vLGN units have a silent suppressive surround. Estimates of the proportion of vLGN units with receptive field surrounds vary widely (27% of the units in Hale and Sefton's study vs. 7% in the study of Sumitomo et al., 1979) Hale and Sefton (1978) noted that the receptive fields of sustained "on" units that changed their firing rates with changes in luminance never had surrounds. Groos and Rusak (1982) concluded that the majority of vLGN neurons projecting to the suprachiasmatic nucleus show these "luminance-coding" responses.
The retinal input to the vLGNe of the rat is visuotopically organized. Conduction velocities of retinal afferents to the vLGN appear to be relatively slow, indicating that retinal afferents are probably originating from retinal ganglion W-cells. Most units are driven only by the contralateral eye.

B.2. Electrophysiology of the vLGN in rabbits

There has been only one report of a survey of visual response types of units in the vLGN in rabbits (Mathers and Mascetti, 1975). In the rabbit vLGN, the nasal visual field is represented dorsally, while the temporal field is represented ventrally. The more lateral regions of the vLGN contain representations of the more dorsal parts of the visual field. Thus, the visuotopic organization of the rabbit vLGN is similar to that of the vLGN of the rat (Nagata and Hayashi, 1984).

Of 109 vLGN units tested for visual responses, 52 units were in the vLGNe and 57 in the vLGNi. In the vLGNi, 76% of the units were responsive to light stimuli, compared with 98% of units in the vLGNe. The observation of visually responsive units in the vLGNi in the rabbit contrasts with studies in the rat, where visually responsive units are reported to be found only in the vLGNe. Approximately 25% of units in both
divisions of the rabbit’s vLGN responded to light, but had no discernible borders to the receptive field area, or in some instances responded only to whole eye illumination. Units with concentric or uniform receptive fields in the vLGN had larger receptive field diameters than did similar units in the dLGN, although the range of receptive field diameters in these two nuclei was similar. A very small number of motion- and direction-sensitive units were found in this study.

In summary, the response characteristics of units in the rabbit vLGN appear very similar to units in the rat vLGN. Studies of the rabbit vLGN indicate (1) the presence of visually responsive units in the vLGNi, and (2) the presence of units with indefinite receptive fields, both of which are not reported for the vLGN of the rat.

B.3. Electrophysiology of the vLGN in cats

The most detailed report of the receptive field characteristics of units in the vLGN of cats was by Spear, Smith and Williams (1977). Receptive field types of 154 units were classed into seven groups.

Group I units (27% of the total sample) had uniform receptive fields. Most of these units (88%) discharged only at light onset. The majority of these units had
transient responses, but 8% produced a sustained discharge throughout the duration (up to 30 s) of light presentation.

Group II units (19%) had concentric receptive fields. Most (97%) of these units responded to light onset only. The majority (76%) of these units had a transient response. Silent suppressive surrounds were found in 70% of the units with concentric receptive fields. The remaining 30% of the units had active surrounds. In these units, simultaneous stimulation of both center and surround resulted in a reduced response compared to stimulation of either region alone. In half of the units, diffuse illumination resulted in no response. In other units the mutual inhibition was only partial, and in these units the center response dominated that of the surround.

Group III units (8%) appeared to signal the level of ambient illumination. These units were characterized by an unusually regular maintained discharge which varied in rate relative to the level of illumination of the receptive field. These units required very large stimuli to respond (typically 10 degrees in diameter or more) and had very large receptive fields (30 degrees in diameter or more) with poorly defined boundaries. None of these units had receptive field surrounds, so that the level of full-field illumination was as effective in
controlling the discharge of the unit as was stimulation of the mapped receptive field. All 12 units increased firing rate to an increased level of illumination.

The remaining classes of response types were more rarely encountered. A contour moving across the receptive field was the optimal stimulus for 4% of the vLGN units. Some (3%) of the vLGN units were sensitive to the direction of stimulus movement through the receptive field.

A relatively large proportion of units (16%) gave indefinite responses to light. Some of these units gave brisk and consistent responses to whole-eye illumination but no receptive field could be localized, while others seemed to have a receptive field but responses were either too weak or too inconsistent to adequately define the boundaries. Finally, 23% of the units gave no response to the light stimuli.

Most vLGN units were driven only by the contralateral eye, but 30% were binocularly driven and 13% were driven only by the ipsilateral eye. A visuotopic organization was noted, with the lower visual field represented anteriorly and the upper visual field posteriorly. It appeared that the vertical meridian was represented along the dorsomedial border of the vLGN while the temporal periphery was represented ventrolaterally. Visual receptive field sizes for vLGN
units were generally larger than those for dLGN units at all visual field eccentricities.

Hughes, with colleagues, has published two reports on the receptive field characteristics of vLGN units in the cat. In the first report (Hughes and Ater, 1977) the receptive field properties of 177 units were classified. The results of this study are summarized in Table 7. The results did not differ substantially from those presented in a second, more complete report (Hughes and Chi, 1983). In this second study, the visual receptive fields of 293 units in the vLGN of the cat were described. The response of all units to light of various wavelengths was assessed with broad band filters (> 600nm, 430 to 460 nm, and 520 to 540 nm) matched for luminance with white light. Receptive field types were classed into 5 groups.

Group I units (47% of the total sample) had uniform or concentric large receptive fields and sluggish firing patterns. Some (38%) of these units responded to movement of the stimulus and 3 units were directionally selective. Of the units that responded best to a stationary stimulus, most (88%) had uniform receptive fields.

Group II units (12% of the total sample) had small concentric (56%) or uniform (44%) receptive fields and brisk responses. All of these units responded solely to
the contralateral eye.

Group III units (11%) had very large receptive fields covering the entire contralateral visual field. These units responded sluggishly and many had a high rate of spontaneous firing. A few units appeared to be luminance-coding cells, similar to those described by Spear et al. (1977).

Group IV units (10%) had uniform receptive fields and sluggish responses, similar to Group I units. These units were distinguished by their differing responses to different wavelengths. Fifteen units responded primarily to blue light (430 to 460 nm). The other units in the group showed opponent color responses. All but one of these units responded with "on" to blue light, "off" to red light and variably to green. A few of each of these categories of wavelength-sensitive units were binocularly driven.

Group V units (20%) did not respond clearly to visual stimuli.

About 20% of all vLGN units in this sample had a demonstrable binocular input. Most responded predominantly to the contralateral eye, although a few units showed an equal response to illumination of either eye. When both eyes were illuminated, these units showed a greater response than to illumination of either eye alone. Units in the vLGN never responded to
illumination of only the ipsilateral eye. It was noted that responses to optic chiasm stimulation had longer latencies in the vLGN relative to those in the dLGN. There was no clear relationship between the receptive field type and the location of the unit within the vLGN.

Hada et al. (1986) used stimulating electrodes to identify neurons projecting to the superior colliculus and pretectum while recording from the vLGN in cats. The receptive field characteristics of vLGN units in this study do not differ from those reported in previous studies except that a very high (58%) percentage of units were not visually responsive. Units were classified as having phasic or sustained visual responses. Very few units had sustained responses (16% of the visually responsive units; 7% of the total sample). The only significant difference between the visual responses of units projecting to the pretectum or superior colliculus and other vLGN units was a smaller percentage of sustained "on" units in the group of projection neurons. Nine units could be antidromically activated from stimulation in both sites, indicating that these neurons projected to both the pretectum and the superior colliculus. A visuotopic organization of receptive fields similar to that described by Spear et al. (1977) was noted. Visually responsive units were located in the external layer of the vLGN. Neurons
activated by pretectal or superior colliculus stimulation were likely to be clustered in the lateral part of the middle section of the rostrocaudal extent of the vLGN.

An opto-vestibular role for the vLGN is suggested by several studies. Responses of vLGN neurons to paired visual and vestibular stimuli was studied in fully awake cats by Putkonen et al. (1973). As cats were rotated, nystagmus and single unit responses in the vLGN were recorded. A total of 83 units were recorded from 3 cats. Twenty of these units, encountered after the electrode had left the area clearly responding to light, showed receptive field characteristics very different from those recorded in the dLGN. Only 3 of these units responded to changes in background illumination ("on" responses in 2 cases, "off" in one case). None had a receptive field or responded to a moving spot. All, however, responded with a change in discharge rate in relation to the rotation of the table. This effect persisted in complete darkness. The firing rate appeared to map the changes in angular velocity. In addition to the tonic directional response, a relationship of firing rate to visual nystagmus was observed in 15 cases. Of the 20 neurons discussed, 17 were clustered within the ventro-medial vLGN.

In a similar study, Magnin and Putkonen (1978)
recorded units in the vLGN of cats during vestibular and optokinetic stimulation. Units were found to respond to horizontal vestibular and/or optokinetic stimulation, many in a direction selective manner. Most of these units did not respond to light. A few units responded to light, but no receptive field could be mapped. Many units fired in fixed relation with saccadic or nystagmic eye movements. Most of these units were located in the medial vLGN (Jordan and Hollander's vLGNa and vLGNb).
Appendix C. The phase response curve

This section will describe how the function relating the phase shift induced by a stimulus to the phase of the cycle at which the stimulus was applied (a phase response curve or PRC) can be used to analyze the process of entrainment of a rhythm to an imposed light:dark (LD) cycle.

A PRC for 15 min light pulses is shown in figure 29A. The phase of the light pulse is given in circadian time (CT) to correct for deviations of the rhythm's period from 24 h. Activity onset is arbitrarily labeled CT12. Delaying phase shifts are observed after light pulses given between CT10 - 14, while advancing phase shifts are seen after light pulses given between CT16 - 20.

Consider the activity rhythm of a hypothetical animal housed in an arrangement such as that diagrammed in figure 29B. This animal has access both to a nest cage, which is always dark, and an outer cage, which is under a 12:12 LD cycle. Activity rhythms of two animals under such housing conditions are shown in Figure 29, C and D. The animal in Figure 29C has a rhythm with an endogenous period less than 24 h. For the first few days in this situation, the animal sees no light and expresses its endogenous rhythm, starting its activity
earlier each day. This pattern continues until day 4, when the animal emerges from the nest cage and is exposed to light in the outer cage at approximately CT12. It quickly returns to the nest cage for a short period and re-emerges later, when the outer cage is dark. Thus, the animal effectively gives itself a brief light pulse at CT12. The effect of a light pulse at CT12 is to delay the rhythm; this can be seen either in the PRC of figure 29A or in the activity rhythm in figure 29C.

This process repeats itself several times in the record shown in figure 29C. The final result is that the animal remains, on average, synchronized with the LD cycle (i.e., its period which, when the sensitive portion of the PRC is in darkness, is less than 24 h, comes to equal 24 h on average). Figure 29D shows how a similar process could entrain an animal with an endogenous period greater than 24 h, with repeated advance shifts causing the long period to shorten, on average, to that of the 24 h lighting cycle.

A more common laboratory situation is that animals are subjected to an LD cycle, but they do not have access to a dark nest cage. Consider the case of a light:dark cycle with a dark period much shorter than 12 h, for example, a 16:8 LD cycle. Nocturnal animals would entrain to this type of LD cycle so that most of
their activity is confined to the dark phase. Since the light-sensitive phase is approximately 10 - 12 h in length and the dark phase is only 8 h long, animals are likely to be exposed to light both during phases at which light delays the rhythm and also during phases at which light advances the rhythm. Stable entrainment would then be observed at a point where the net effect of the delaying and advancing phase shifts which the animal experiences daily would equal the difference between the animals endogenous period and the period of the imposed cycle.

The steady-state phase of entrainment would, therefore, vary systematically as the difference between the endogenous period and the entraining signal is varied. The significance of the PRC is that it permits strong (and empirically verified) predictions of the phase of entrainment under a variety of entraining cycles. (Pittendrigh and Daan, 1976b). The biological significance of the PRC derives from the fact that the phase of entrainment of an animal's activity determines when the animal will perform some critical biological function, including food gathering, mating, etc. The daily phase at which these activities are performed is a critical determinant of their likelihood of success. The PRC is, therefore, a measure of a mechanism of far-reaching ecological and physiological importance.
Figure 29. (A.) A light pulse phase response curve. (B.) A schematic diagram of an experimental setup involving a nest cage in constant darkness and an outer cage under a light:dark (LD) cycle. (C.) An example of an activity record of an animal with an endogenous period shorter than 24 h under the housing conditions shown in (B). (C.) An example of an activity record of an animal with an endogenous period greater than 24 h under the housing condition shown in (B).
REFERENCES


Colmers, W.F., Lukowiak, K. and Pittman, Q.J., Neuropeptide Y reduces orthodromically evoked population spike in rat hippocampal CA1 by a possibly presynaptic mechanism, Brain Research, 346 (1985) 404-408.


DeValois, R.L., Color vision mechanism in the


ascending from the midbrain central gray, and from the region lateral to it, in the rat, J. Comp. Neurol., 241 (1985) 285-310.


Emson, P.C. and DeQuidt, M.E., NPY - a new member of the pancreatic polypeptide family, Trends in Neurosci.


Fuller, J.H. and Schlag, J.D., Determination of the antidromic excitation by the collision test: Problems of interpretation, Brain Research, 112 (1976) 283-298.


Groos, G. and Hendriks, J., Circadian rhythms in


Holcombe, V. and Guillery, R.W., The organization of


Hoover, D.B. and Jacobowitz, D.M., Neurochemical and histochemical studies of the effect of a lesion of the nucleus cuneiformis on the cholinergic innervation of discrete areas of the rat brain, Brain Research, 170 (1979) 113-122.


Hsu, S.M., Raine, L. and Fanger, H., Use of

Hughes, C.P. and Ater, S.B., Receptive field properties in the ventral lateral geniculate nucleus of the cat, Brain Research, 132 (1977) 163–166.


Inouye, S.T., Light responsiveness of the suprachiasmatic nucleus within the island with the retino-hypothalamic tract spared, Brain Research, 294 (1984) 263–268.


Kawamura, S., Sprague, J.M. and Niimi, K., Corticofugal projections from the visual cortices to the thalamus, pretectum and superior colliculus in the cat,

Kicliter, E. and Bruce, L.L., Ground squirrel ventral lateral geniculate receives laminated retinal projections, Brain Research, 267 (1983) 340-344.


Klein, D.C. and Moore, R.Y., Pineal N-acetyltransferase and hydroxindole-O-methyltransferase: Control by the retinohypothalamic tract and the suprachiasmatic nucleus, Brain Research, 174 (1979) 245-262.


Legg, C.R., Effects of subcortical lesions on the
pupillary light reflex in the rat, Neuropsychologia, 13 (1975) 373-376.


Legg, C.R., Visual discrimination impairments after lesions in zona incerta or lateral terminal nucleus of accessory optic tract, Brain Research, 177 (1979b) 461-478.


Mathers, L.H. and Masetti, G., Electrophysiological
and morphological properties of neurons in the ventral lateral geniculate nucleus of the rabbit, Exp. Neurol., 46 (1975) 506-520.


Mesulam, M.-M., Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinegenic blue reaction-product with superior


Montero, V.M., Brugge, J.F. and Beitel, R.F., Relation of the visual field to the lateral geniculate body of the albino rat, J. Neurophysiol. 31 (1968) 127-134.

Moore, R.Y., Retinohypothalamic projection in mammals: a comparative study, Brain Research, 49 (1973) 403-409.

Moore, R.Y. and Eichler, V.B., Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat, Brain Research, 42 (1972) 201-206.


O'Donohue, T.L., Chronwall, B.M., Pruss, R.M., Mezey, E., Kiss, J.Z., Eiden, L.E., Massari, V.J.,

Ohara, P.T., Lieberman, A.R., Hunt, S.P. and Wu, J-Y., Neural elements containing glutamic acid decarboxylase (GAD) in the dorsal lateral geniculate nucleus of the rat; immunohistochemical studies by light and electron microscopy, Neuroscience, 8 (1983) 189-211.


Pasquier, D.A. and Villar, M.J., Specific serotonergic projections to the lateral geniculate body from the lateral cell groups of the dorsal raphe nucleus, Brain Research, 249 (1982b) 142-146.


Pickard, G.E., The afferent connections of the


Pittendrigh, C.S. and Daan, S., A functional
analysis of circadian pacemakers in nocturnal rodents.

1. The stability and lability of spontaneous frequency,
   J. Comp. Physiol., 106 (1976a), 223-252


   Putkonen, P.T.S., Magnin, M. and Jeannerod, M., Directional responses to head rotation in neurons from the ventral nucleus of the lateral geniculate body, Brain Research, 61 (1973) 407-411


Rusak, B., Involvement of the primary optic tracts in mediation of light effects on hamster circadian rhythms, J. Comp. Physiol., 118 (1977) 165-172.


Schwartz, W.J., Davidson, L.G. and Smith, C.B., In vivo metabolic activity of a putative circadian


Sumitomo, I., Ide, K., Iwama, K. and Arikuni, T., Conduction velocity of optic nerve fibers innervating

Sumitomo, I., Nakamura, M. and Iwama, K., Location and function of the so-called interneurons of the rat lateral geniculate body, Exp. Neurol., 51 (1976) 110-123.


Trejo, L.J. and Ciferri, C.M., Cells in the pretectal olivary nucleus are in the pathway for the direct light reflex of the pupil in the rat, Brain Research, 300 (1984) 49-62.

Updyke, B.V., Topographic organization of the projections from cortical areas 17, 18 and 19 onto the thalamus, pretectum and superior colliculus in the cat, J. Comp. Neurol., 173 (1979) 81-122.

Van den Pol, A.N. and Powley, T., A fine-grained

