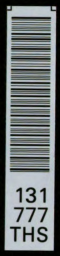

This is to certify that the

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COMPARATIVE ANATOMY OF THE RETINO-HYPOTHALAMIC TRACT IN RODENTS

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Timothy Grant Youngstrom

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of the requirements for

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Major professor

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COMPARATIVE ANATOMY OF THE RETINO-HYPOTHALAMIC TRACT IN RODENTS.

By

Timothy Grant Youngstrom

A THESIS

Submitted to
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ABSTRACT

COMPARATIVE ANATOMY OF THE RETINO-HYPOTHALAMIC TRACT IN RODENTS.

By

Timothy Grant Youngstrom

Several mammalian species reproduce during specific periods annually. These animals utilize photoperiod (i.e., day length) to indicate optimal times and are referred to as photoperiodic. Previous reports indicate that the pattern of retinal input to the suprachiasmatic nuclei (SCN) of the hypothalamus is more symmetrical bilaterally, following unlateral intraocular injection of HRP, in the photoperiodic hamster than in several non-photoperiodic mammalian species. In this study, male rodents were injected with horseradish peroxidase (HRP) intraocularly to trace the retino-hypothalamic tract (RHT) in photoperiodic and non-photoperiodic species. Termination of the RHT in photoperiodic Turkish hamsters is similar to the pattern in golden hamsters. Two photoperiodic species of mice and non-photoperiodic house mice display patterns of termination similar to the non-photoperiodic rat. Symmetrical retinal input to the SCN is not a universal feature of photoperiodic rodents. Other features of the hypothalamus are discussed as possible neural substrates involved in responses to photoperiod.

ACKNOWLEDGMENTS

The assistance provided by Dan Hood, Linda Moskwa, Robert Ryan, Dawn Langsbury , Susan Olson and Beth Dettmer in the collection of data for this paper and technical advice given by Ken Smithson and Dr. C. Sisk are gratefully acknowledged. The advice and guidance during this study by Drs. J. Zacks and M. Rilling have been appreciated. The assistance, advice and facilities of Dr. A.A. Nunez provided during this project are deeply appreciated. (Supported in part by NIMH grant MH 37877 to A.A.N.)

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LIST OF ABBREVIATIONS

BIL-Bilateral

C-Coronal

H-Horizontal

III-Third Ventricle

L-Animal's Left

L:D-Light:Dark

OC-Optic Chiasm

ON-Optic Nerve

OT-Optic Tract

PVN-Paraventricular Nuclei of the Hypothalamus

RHT-Retino-Hypothalamic Tract

SCN-Suprachiasmiatic Nuclei of the Hypothamalus

SON-Supraoptic Nuclei of the Hypothalamus

TX-Transected Optic Nerve

TMB-3,3',5,5'-Tetramethylbenzidine

UNI-Unilateral

3V-Third Ventricle

INTRODUCTION

CIRCADIAN RHYTHMS

Regularly reoccurring behavioral and physiological events of about (circa) one day (dies) have been described in many animals. A wide variety of behaviors exhibit rhythmic patterns, but care must be taken not to confuse an event that is generated by the organism with one that is the result of an external event (for review see Bunning, 1973; Rusak and Zucker, 1979). A true circadian rhythm persists despite a constant external environment, as in conditions of constant light and free access to food and water.

To be a circadian rhythm does not, however, imply that the observed pattern of activity must be "blind" to external environmental changes. As a matter of survival it is important that the animal be able to identify regular events in the environment and to respond in a manner most appropriate to these regular changes. An external event may result in an alteration either in the pattern or intensity of activity shown by the animal. Typical circadian rhythms in behavior such as activity and feeding rhythms are sensitive to changes in the environment. For example, an external cue, a Zeitgeber (time giver), can entrain the circadian rhythm to itself providing the environmental event reoccurs about

every 24 hours. The daily change in the environment from light to dark is the most effective Zeitgeber for the entrainment of behavioral rhythms in mammals.

PHOTOPERIODISM

The reproductive cycle is an example of both physiological and behavioral activities which are in part influenced by external environment. Female rats (Rattus norvegicus), for example, are nocturnal animals which, when exposed to constant dark in their environment exhibit an estrous or ovulatory cycle which is shorter than the normal 4-day cycle seen when the animals are exposed to a light-dark cycle. Similar results are seen in the mouse (Mus musculus) (Campbell, Ryan and Schwartz, 1976). Regardless of the ratio of light to dark (L:D) rats and house mice do not exhibit an annual or seasonal rhythm in breeding behavior or reproductive physiology; they do not display a photoperiodic response. Females of these species have a regular 4-day estrous cycle that is maintained throughout the year. Males of these species similarly maintain reproductive competence regardless of photoperiodic length (Campbell, et al., 1976). Such species are called "Non-photoperiodic" (Bunning, 1973; Reiter, 1980a).

In contrast, a wide variety of animals demonstrate distinctive annual breeding seasons. Examples include sheep, deer, hamsters and deer mice. In these species changes can

be observed in activity patterns and physiology which are directly related to reproduction (Reiter, 1980b; Stetson & Watson-Whitmyre, 1976; Turek & Campbell, 1979). During the breeding season the adult male golden hamster's testes are of mature size. At predictable times in the year the testes regress and gonadal and gonadotropin hormone levels fall. After a period of months, the testes spontaneously recrudesce, gonadotropin and gonadal hormonal levels rise, and the animal is now ready to breed.

Female hamsters respond to short photoperiod length (less than 12.5 hrs) by exhibiting continuous diestrous. When exposed to long photoperiods, female hamsters begin to display a 4-day estrous cycle (Stetson & Watson-Whitmyre, 1976). Regression and recrudescence are predictable based upon the ratio of light to dark in the environment or whether the animal has been recently exposed over a number of circadian cycles to light pulses during the light sensitive portion of the endogenous circadian rhythm (Elliott & Goldman, 1981). Those animals exhibiting similar sorts of anatomical and physiological changes are called "Photoperiodic".

SUPRACHIASMATIC NUCLEI

Virtually all animals studied to date use the light-dark cycle as a reference to which circadian rhythms

are entrained (Hoffmann, 1981; Rusak & Zucker, 1979). In the case of mammals, information is received by the retina and transmitted in the optic nerve by the retino-hypothalamic tract (RHT) to the suprachiasmatic nuclei (SCN). The RHT was first described in the rat by Moore and Lenn (1972). In that study an autoradiographic technique was used to trace the axons from the retina to SCN. The examination revealed concentrations of tritiated amino acid, interpreted as being axon terminals within the SCN. The heaviest concentrations were in the ventro-caudal portions of the SCN, with the nucleus contralateral to the injected eye receiving approximately twice as many axon terminals as the nucleus ipsilateral to the injection.

The SCN are believed to be the anatomical substrate for a circadian pacemaker (Moore & Klein, 1974; Moore, 1978). Lesion studies have shown that destruction of the SCN results in loss of nearly all rhythmic behavior in rats and hamsters (Klein & Moore, 1979; Moore & Eichler, 1972; Stephan & Zucker, 1972; Rusak, 1977; Stetson & Watson-Whitmyer, 1976). Similar studies in which lesions were placed adjacent to the SCN have not duplicated the lesion effects. However, combined knife cuts placed caudal, dorsal and lateral to the SCN damage neural connections to other areas of the brain from the SCN and result in circadian acyclicity (Moore & Klein, 1974; Inouye & Kawamura, 1979). Finally, while lesions in other regions of the brain may produce alterations or loss of oscillation in a few rhythms,

the results are never observed to be as wide spread as when the SCN are destroyed. The data collected so far indicate that the SCN are at least the major source of rhythmic input to the rest of the brain.

The role of the SCN in the reproductive photoperiodic response in Syrian hamsters is not clear. Bilateral enucleation of male golden hamsters results in gonadal regression within 9 weeks (Rusak & Morin, 1976). If the SCN, and thus the RHT, are destroyed it might be predicted that the animal would essentially be exposed to continuous dark since information regarding the environmental light/dark cycle would have been lost. Yet when Syrian hamsters that have been exposed to long photoperiods receive SCN lesions followed by exposure to short photoperiods, the short-day response does not occur. In males the testes are maintained (Rusak & Morin, 1976) and the lesioned females display persistent estrus rather than diestrous (Stetson & Watson-Whitmyre, 1976). The SCN not only provide information regarding the location of the light exposure within the circadian cycle and entrainment of the cycle to that light, but also play an important role in mediating the photoperiodic reproductive response (Turek & Campbell, 1979).

HORSERADISH PEROXIDASE AS A TOOL TO STUDY RETINAL PROJECTIONS

Recently, the enzyme horseradish peroxidase (HRP) has been introduced as a tool to trace neural tracts (Kristensson, Olsson and Sjostrand, 1971; LaVail & LaVail, 1972). In the process of anterograde axonal transport HRP is taken up by pinocytosis and sequestered within membrane bound organelles. The organelles are then carried by an active transport mechanism within the axonal agranular reticulum (Colman, Scalia & Cabrales, 1976). This technique involves reacting the tissue containing HRP with a chromogen, such as benzidine or one of its derivatives (i.e.-diaminobenzidine [DAB], 3,3',5,5'-Tetramethylbenzidine [TMB]). Use of the HRP-TMB procedure has proven highly sensitive and yet does not require a large investment of time before results can be evaluated. In the rat, however, the HRP-TMB technique has been used to look specifically at the RHT in only 2 Sprague-Dawley rats (Pickard & Silverman, 1981). This was done in order to examine the possibility that there exists a more extensive, though perhaps diffuse, pattern of RHT termination within the dorsal SCN. While HRP has been used in other studies to trace RHT termination in the rat SCN, the less sensitive chromogens benzidine and DAB have been used (Colman, *et al.*, 1976; Kita & Oomura, 1982).

Several investigations of the RHT pattern of innervaton within the SCN of the golden hamster (*Mesocricetus auratus*)

have recently been conducted (Pickard & Silverman, 1981; Pickard, 1982). In this species a distinct difference appears in RHT termination when compared to the rat. In the golden hamster termination of the RHT in the SCN appears more pervasive bilaterally and nearly symmetrical following a unilateral injection of HRP when examining the SCN rostrally to caudally. However, these species comparisons were made with data obtained from only 2 rats or using data obtained with significantly different techniques of detecting tract termination and so, observed differences may be a matter of technique sensitivity rather than species differences. In reports by Pickard (1982) and Pickard and Silverman (1981) it was suggested that the different patterns observed for the rat and hamster may be characteristic of non-photoperiodic versus photoperiodic species respectively. However, the reports on non-photoperiodic species cited to support the suggestion that patterns of RHT termination may be different in photoperiodic versus non-photoperiodic species discuss the SCN only in passing or use autoradiographic techniques rather than an HRP technique (Drager, 1974; Tigges, Bos & Tigges, 1977). Therefore, there is a distinct lack of information about the comparative anatomy of RHT and the SCN. Furthermore, in order that more reliable comparisons may be made, previous studies should be repeated using the more sensitive HRP-TMB techniques available.

In summary, despite the lack of data available for precise comparisons, there may be species differences with respect to pattern and symmetry of innervation of the SCN by the RHT. For example, investigation of retino-fugal pathways using unilateral injections of HRP has led to the identification of differences in retino-hypothalamic terminals in photoperiodic vs non-photoperiodic species of rodents. These differences in innervation may be the source of differential response to the photic input. Thus, in the photoperiodic golden hamster the RHT invades more of the dorso-lateral and lateral aspects of the SCN, whereas, in the non-photoperiodic rat, the retinal input is restricted to the ventro-lateral SCN. Furthermore, the asymmetrical input reported for the rat (i.e., 2:1 in favor of the contralateral SCN) is not seen in the hamster.

In this study, the the pattern of termination made by RHT fibers in the SCN of non-photoperiodic house mice was compared to that of Turkish hamsters, a photoperiodic species (Hall, Bartke & Goldman, 1982). Two additional photoperiodic species (deer mice [Peromyscus maniculatus bairdi] and white-footed mice [Peromyscus leucopus]) were examined with the prediction made that the pattern of termination would be similar to that of the photoperiodic Turkish hamsters.

METHOD

Intraocular Injections

Adult male Turkish hamsters (Mesocricetus brandti), house mice (Mus musculus), deer mice (Peromyscus maniculatus bairdi) and white-footed mice (Peromyscus leucopus) were used (see Table 1). Injections of 30%-50% (w/v) HRP (Sigma Type VI) within the vitreous of the eye were made in animals anesthetized with Equithesin (4.5 ml/kg). Following a survival period of 24 hours the animals were again deeply anesthetized and the upper part of the body perfused using a transcardial technique (after Mesulam, Barbas, Carson, Gowan, Knapp, Moss and Mufson, 1980; Rosene & Mesulam, 1978). After exposing the heart an intracardial bolus of 20 U heparin in 1 ml physiological saline was given. A cold ($4^{o}C$) 5.0% (w/v) solution of sodium chloride containing heparin (1000 U/liter) was first passed through the systemic circulation to remove blood. This was followed by cold 4.0% (v/v) gluteraldehyde in 0.1 M sodium phosphate buffer (pH-7.4) fixative solution. The brains were removed and kept refrigerated in sucrose solutions of increasing concentration (steps of 5, 10, 15 and 20%) over a 48 hr period. To improve fixation, a small amount (approximately 10 ml) of

TABLE 1. NUMBER OF CASES USED FOR EACH SPECIES*

SPECIES	INTRAOCULAR INJECTION (INTACTS) UNI	INTRAOCULAR INJECTION (INTACTS) BIL	BIL. OPTIC NERVE TX	INTRAVENOUS§ INJECTION
Turkish Hamster	5-C 1-H	2-C 0-H	0	0
House Mouse	20-C 4-H	10-C† 0-H	4	24
Deer Mouse	13-C 3-H	17-C 2-H	5	22
White-Footed Mouse	8-C 1-H	2-C 1-H	5	21

*C=Coronal plane of sectioning; H=Horizontal plane of sectioning.

§Survival time 1 hr (n=9), 2 hr (n=9), 4 hr (n=9), 8 hr (n=9), 16 hr (n=9), 20 hr (n=9), 24 hr (n=13).

†3 house mice and 6 deer mice included here received intraocular injection of 50% HRP. All other cases received injections of 30% HRP.

4.0% gluteraldehyde was added to the 5% sucrose (approximately 50 ml).

Brains were then frozen on the stage of a freezing microtome and sectioned, either coronally or horizontally, at 40 µm (Turkish hamsters) or 50 µm (other species) into cold 0.1 M sodium phosphate buffer (pH-7.4) on ice. Once all required sections had been collected the sectioned tissue was incubated with a solution containing TMB then reacted with the TMB by the introduction of a 0.3% (v/v) solution of hydrogen peroxide. Reacted sections were then mounted to gelatinized slides and allowed to dry overnight. The sections were counterstained with either Pyronin Y to reveal the cell's limiting membrane or cresyl violet to demonstrate cell nuclei and Nissl substance. Slides were finally covered with coverglass and examined microscopically under darkfield illumination.

Analysis of Tissue

Tissue was examined under darkfield illumination to determine the quality of the histology and the presence or absence of HRP reaction product. This reaction product has distinctive shape and color characteristics which permit relative ease in distinguishing it from histological and chemical artifacts. With the aid of a camera lucida attachment, drawings were made to determine the location of the SCN in relation to the remainder of the hypothalamus and to

reveal the overall shape to the nuclei in each species.

CONTROL PROCEDURES

Bilateral Optic Nerve Transections. As a control for uptake of HRP from the general circulation by hypothalamic neurons following intraocular injections of HRP, bilateral optic nerve transections were performed in several cases (See Table 1). Male deer mice and house mice were anesthetized as described previously. Following the method described by Richter (1976) the connective tissue was separated from the point of attachment to the eye ball either from the rostral or caudal pole. Then either the lateral or medial rectus muscles were transected. The eye was rotated so as to reveal the rear of the socket and the optic nerve. The optic nerve was located and transected avoiding damage to adjacent tissue and the blood vessels surrounding the optic nerve. The eye was returned to a normal position and the lids of the eye sutured closed. Following a 13-day recovery period and to allow degeneration of optic nerve fibers, the animal received bilateral intraocular injections of HRP as described above. Tissue was prepared as reported above.

Intraveneously Injected HRP. Male deer mice, white-footed mice and house mice were anesthitized with Equithesin and the right common iliac vein was visualized external to the abdominal wall for approximately 5 mm distal. A 5 cm cannula was made from Intramedic PE-20 tubing (Becton,

Dickinson and Co., Parsippany, NJ). A tapered end was produced by heating the tubing and pulling the ends apart as it cooled. A scalpel blade was used to cut the tapered region in half transfersely and a bevel was cut at the end of the tapered orifice. The opposite end was slipped over the needle of a 10 microliter Hamilton syringe needle and the assembly was filled with 50% HRP (w/v) in distilled water to deliver 9 microliters.

A nick was cut into the wall of the vein into which the cannula was introduced. Once it was determined that the liquid from the cannula would flow freely into the vein the HRP was delivered into the circulation. The cannula was then immediately withdrawn and a 5 mm cube of Gelfoam (Upjohn, Kalamazoo, Mich.) was applied over the vein and held in place against the cut with finger pressure for one minute. The incision was sutured closed in the case of animals which were to survive more than eight hours otherwise the hind quarters of the animal were encased in tape to hold the wound closed until the time of perfusion. Survival periods were 1, 2, 4, 8, 12, 16, 20 or 24 hours. Three animals were assigned to each survival period from each species of mice. Tissue was prepared as has been previously described above.

RESULTS

INTRAOCULAR INJECTIONS

TURKISH HAMSTER (*Mesocricetus brandti*)

Retinal Input to SCN. The overall appearance of retinal input to the SCN of the Turkish hamster is similar to that previously reported in the golden hamster (Pickard, 1982; Pickard & Silverman, 1981) after unilateral injections. Throughout the rostro-caudal extent of the SCN the density and location of reaction product is nearly symmetrical bilaterally. In horizontal sections fibers destined to enter the ipsilateral SCN were observed to either approach the ventral border of the nucleus near the midline, or actually cross the midline. Many fibers that had crossed the midline turned slightly rostrally into the contralateral optic nerve before turning back upon themselves and recrossing the midline to finally terminate in the ipsilateral SCN (Figure 1). As a result of this path the pattern of reaction product observed about the ventral border of the ipsilateral SCN was one in which the ventro-medial border displayed a higher density of reaction product than the ventro-lateral boundary. On the other hand those fibers directed toward the

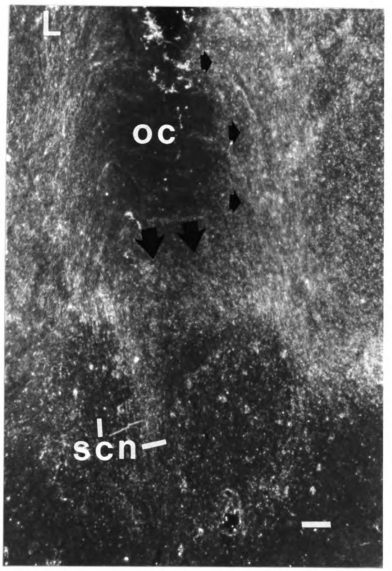

FIGURE 1. Darkfield photomicrograph of a horizontal section from a Turkish hamster brain showing RHT fibers in optic chiasm (small arrows) and innervating SCN. The left eye (upper left corner) was injected with HRP. Many fibers projecting to the ipsilateral SCN cross the midline (large arrows) before recrossing and terminating in the nucleus (Bar=100 μm).

contralateral SCN tended to take a more direct path gathering at a more directly ventral location beneath the SCN.

The rostral 200-300 μm of the SCN tend to contain little or no reaction product. Those granules detected were generally located just dorsal to the ventral boundary (Figure 2). In the middle one third of the SCN rostrocaudally the amount and location of reaction product changed rapidly. Initially the area observed to contain reaction product expanded to include the entire SCN. Density of reaction product remained generally uniform throughout the nuclei. Over the next 100-150 μm the amount of reaction product increases in density with the maximum concentration of product found in the ventro-lateral two-thirds of the nucleus bilaterally (Figure 3). The lowest density of granules was located in a crescent at the dorso-medial boundary against the third ventricle. The level of reaction product decreased in the region immediately adjacent to the ventral border. In the caudal one-third of the SCN the density of reaction product fell although the locations within the nuclei remained generally the same. Bilateral injections of HRP revealed little increase in area containing granules, while the numbers of granules observed did increase.

Possible Retinal Input to Other Hypothalamic Nuclei. Dorsal to the caudal half of the SCN single granules and short chains of granules were observed in most cases. While chains of granules, suggestive of axons, were not long

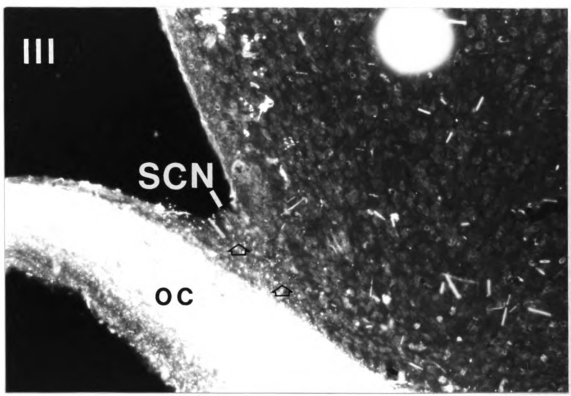

FIGURE 2. Coronal section of the ventral hypothalamus from a Turkish hamster. The left eye was injected with HRP and the eye would be located on the observer's right. Granules of reaction product representing RHT input to rostral SCN are located in ventral SCN (open arrows) just dorsal to the optic chiasm. (Darkfield illumination; Bar=50 μm).

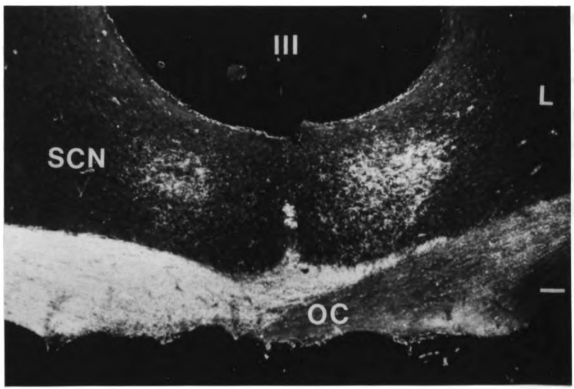

FIGURE 3. Unilaterally injected HRP in left eye of a Turkish hamster demonstrating more nearly symmetrical RHT input to SCN. The caudal region of the nucleus ipsilateral to the injected eye displays a higher concentration of RHT input. (Coronal section; Darkfield illumination; Bar=50 μm).

enough to clearly demonstrate an input to the paraventricular nuclei, the orientation and location of these granules suggest that such a connection is possible (Figure 4). In addition, granules were occasionally observed within the PVN. Similarly, granules were observed in the area dorsolaterally to the SCN.

Inspection of the region including the supraoptic nuclei (SON) revealed reaction product within and dorsal to the contralateral SON in the case of several unilateral injections (Figure 5). The reaction product within the nucleus appeared to be primarily single granules and not fibers passing through to other regions. Those granules found dorsal to the SON appeared to represent fibers. Many of these fibers passed close enough to the border of SON to perhaps make synapses with cells of these nuclei. A few of these fibers may have entered the SON although chains of reaction product representative of processes were not of sufficient length to clearly demonstrate this.

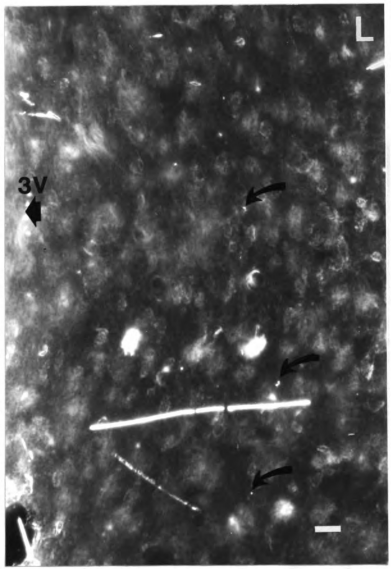

FIGURE 4. Fibers of the RHT project dorsally beyond the boundary of SCN bilaterally. Coronal section of the brain from a Turkish hamster shows a low density of reaction product (arrows) in the hypothalamus. (Darkfield illumination; Bar=20 μm).

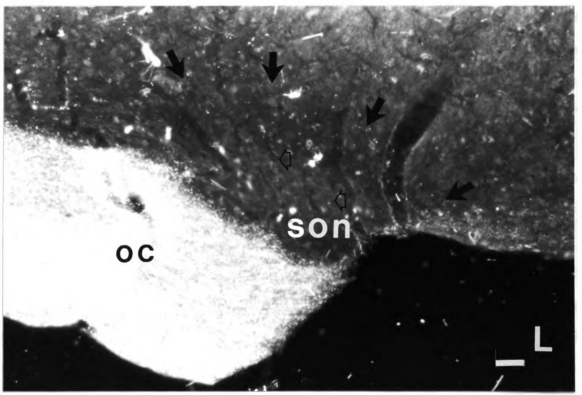

FIGURE 5. Retinal fibers (arrows) dorsal to the supraoptic nucleus labelled after intraocular injection of HRP. Chains of reaction product indicate the location of fibers found dorsal to SON in a brain from a Turkish hamster. Isolated granules in or near SON (open arrowheads) are observed as well. (Darkfield illumination; Coronal section; Bar=50 μm).

DEER MOUSE (*Peromyscus maniculatus bairdi*)

Retinal Input to SCN. In those cases where unilateral injections were administered, the overall retinal input appeared to be asymmetrical with the contralateral SCN displaying a higher density of reaction product (Figure 6). As in the Turkish hamster, the rostral 200-300 μm of the SCN did not show appreciable amounts of reaction product and that which was observed tended to be confined to the ventral boundary of the SCN. In the following 100-200 μm of the SCN there was a rapid increase in concentration of, and area containing, retinal input. As the density increased in this region there was a noticable increase in concentration of granules in the contralateral SCN.

Reaction product in the ipsilateral SCN tended to form a somewhat columnar or crescent shape region of higher concentration relative to the rest of that SCN (Figure 7). This area of high concentration was located in the lateral one-third to one-half of the nucleus. In the case of the contralateral SCN the region of high concentration was found more in the mid-ventral region of the SCN and took a circular form. A region of lowest reaction product density was noted in the dorso-medial SCN bilaterally. At the level of the caudal one-third of the SCN density declined although initially the difference in concentration remained. Near the caudal edge of the SCN a difference in density was not discerned either within or between the nuclei.

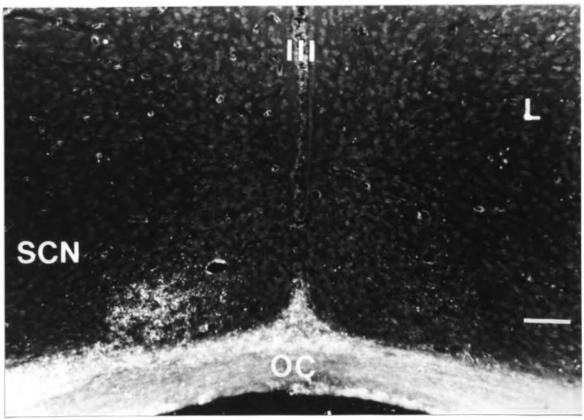

FIGURE 6. The contralateral SCN receives a higher concentration of retinal input as shown following an HRP injection into the left eye of a deer mouse. (Coronal section; Dark-field illumination; Bar=50 μm).

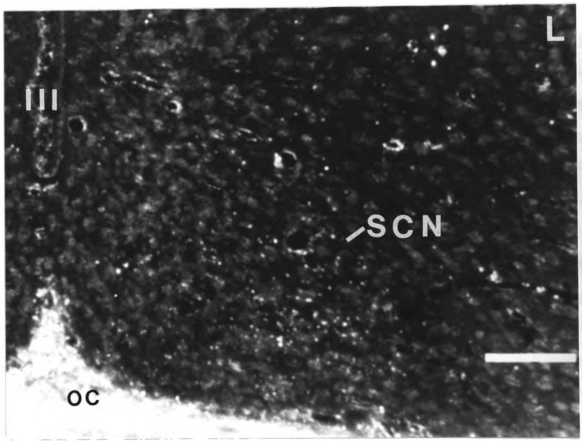

FIGURE 7. Enlargement of Figure 6, demonstrating the ipsilateral nucleus and the patten of retinal input. The dorso-lateral, lateral and ventral areas of the nucleus receives a higher concentrations of RHT input than the dorsal and dorso-medial regions. The boundary of the nucleus is represented by the white line. (Coronal section; Dark-field illumination; Bar=20 μm).

Possible Retinal Input to Other Hypothalamic Nuclei. As in the Turkish hamster, granules of reaction product were observed dorsal and dorso-lateral to the SCN. Some of these granules were close enough to the PVN to suggest that a retino-paraventricular connection is possible. Likewise, in a few cases chains of granules believed to be fibers were observed dorsal to the SON, and others appeared to enter the SON directly from the optic tract.

In an attempt to clarify the issue of direct retinal input to the PVN and SON, 3 house mice and 6 deer mice received bilateral intraocular injections of 9-12 µl of 50% (w/v) HRP in distilled H_2O. In deer mice additional fibers were observed in the regions dorsal to SCN and ventral to PVN and dorsal to SON. However, it appeared that some of the cells within the PVN and SON contained moderate amounts of reaction product (Figure 8). Further, a few processes could be traced back to these cells due to the reaction product they contained (Figure 9). Some of these processes were in such a location as to make it conceivable that what had been interpreted as RHT fibers passing through SCN towards PVN or retinal fibers directed at SON may, in fact, have been processes of these cells passing to the retina. Alternatively, these cells might have taken up HRP, from nearby capillaries, that had leaked into the systemic circulation following intraocular injections. It is not possible at this time to make a clear determination regarding the possibility that the observed processes are retinal in origin and are

FIGURE 8. Reaction product is found in magnocellular neurons (asterisks) of PVN (upper panel) and SON (lower panel) following high concentration and volume injection of HRP to both eyes of a deer mouse. (Coronal section; Dark-field illumination; Bar=20 μm).

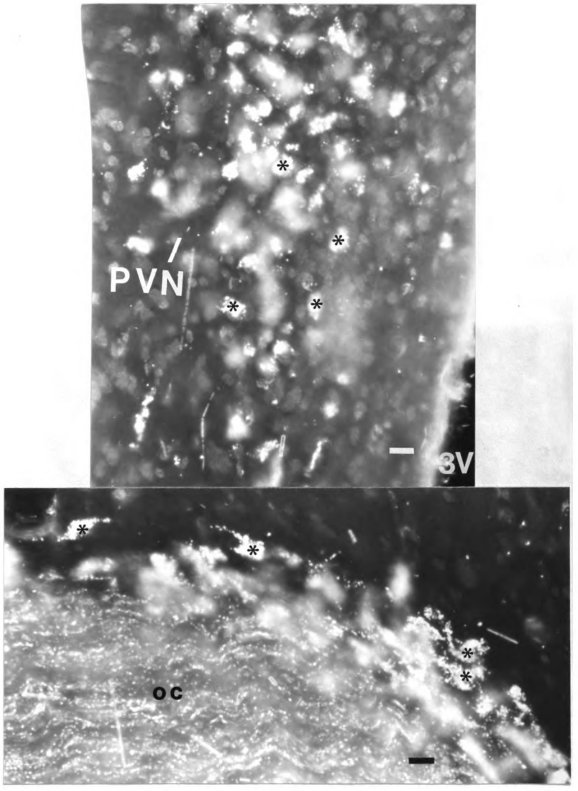

Figure 8.

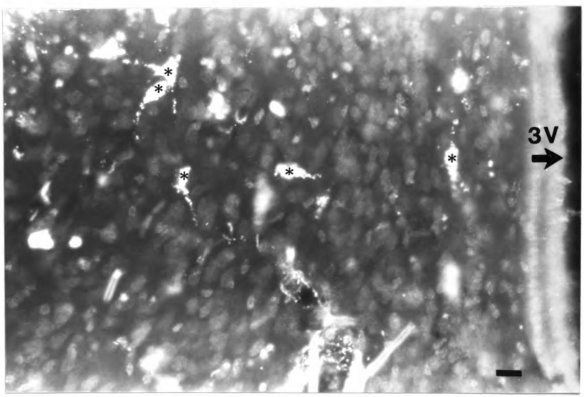

FIGURE 9. Magnocellular components (asterisks) and associated processes in the hypothalamus of a deer mouse are labelled by HRP after bilateral intraocular injections of increased volume and concentration. (Coronal section; Dark-field illumination; Bar=20 μm).

synapsing profusely upon the cells observed or that the processes originate at the cells noted. In one case (84-D6A) there is present a band of fibers within the lateral edge of the optic tract that appears to enter the SON and give off processes into that nucleus (Figure 10).

WHITE-FOOTED MOUSE (*Peromyscus leucopus*)

Retinal Input to SCN. The rostral one half of the SCN in this species displayed minimal reaction product. As in the previously described species there was a rapid rise in observed reaction product over the following 200 μm. Unilaterally injected animals displayed a greater increase in density in the contralateral SCN. Bilaterally the region of high concentration was found in the ventro-lateral quadrant (Figure 11). Reaction product was observed in the entire SCN but was lowest or absent in a crescent of the dorso-medial SCN. The pattern and location of reaction product in the white-footed mouse was quite similar to that of the deer mouse following unilateral injection. In the SCN contralateral to the injected eye, the granules tended to occupy a slightly more lateral location relative to those of the ipsilateral SCN. Caudal SCN displayed a decline in reaction product so that at the level of the caudal boundary there was a nearly equal concentration of reaction product bilaterally.

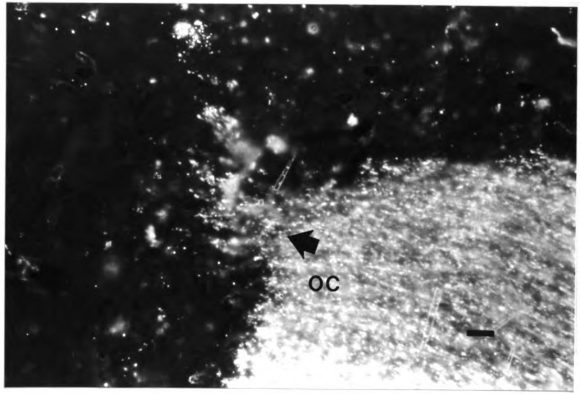

FIGURE 10. Processes labelled by HRP that are located dorsal to SON (arrowheads) in the brain of a deer mouse. A few fibers appear to enter SON (arrow). (Coronal section; Darkfield illumination; Bar=20 μm).

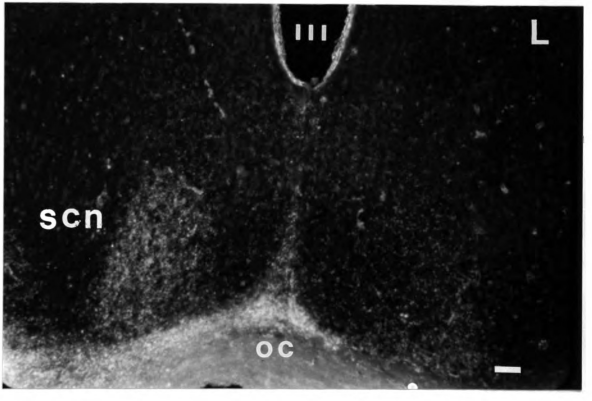

FIGURE 11. A coronal section taken from the brain of a white-footed mouse displays an asymmetrical pattern of RHT input to the SCN. The contralateral nucleus contains a higher concentration of RHT input following unilateral injection of HRP into the left eye. (Darkfield illumination; Bar=50 μm).

Possible Retinal Input to Other Hypothalamic Nuclei. As noted in the previously described species, there was a group of granules dorsal and dorso-lateral to the SCN bilaterally. This reaction product was most apparent at the level of caudal half of SCN (Figure 12). In coronal sections, these granules were observed as far dorsal as the ventral boundary of PVN and as far dorso-laterally as the fornix. Granules forming chains believed to be axons tended to form narrow bands dorsal to the SON. Only occasional granules were noted within the SON proper in this species.

HOUSE MOUSE (Mus musculus)

Retinal Input to SCN. As was described in the photoperiodic species, the rostral 200-300 µm of the house mouse SCN contained minimal amounts of reaction product if any. The next 100-200 µm of the SCN displayed a rapid rise in concentration that initially included the entire nucleus bilaterally (Figure 13). In the following 100 µm an increase in reaction product within the contralateral SCN over the ipsilateral nucleus was observed. The location of highest density was similar to that reported in both species of Peromyscus although the area of generally high reaction product concentration appeared larger (Figure 14). Similarly, the dorso-medial area of each nucleus contained a minimal amount of reaction granules compared to other regions of

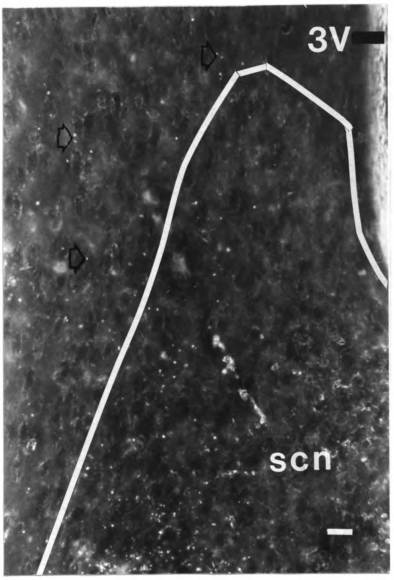

FIGURE 12. Evidence of retinal input (open arrows) to areas of the hypothalamus dorsal to the boundary of the SCN (white line) in a white-footed mouse. (Coronal section; Darkfield illumination; Bar=20 μm).

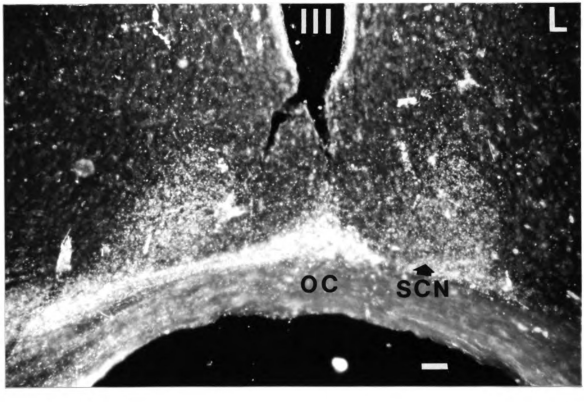

FIGURE 13. A coronal section from the middle 1/3 of the SCN of a house mouse displays approximately symmetrical input of RHT fibers bilaterally at this level of the SCN. Unilateral injection of HRP into the left eye. (Darkfield illumination; Bar=50 μm).

the nuclei. In the caudal one quarter of the SCN the level of reaction product also declines in this species and is nearly equal bilaterally at the caudal pole.

Possible Retinal Input to Other Hypothalamic Nuclei. Granules were also seen dorsal and lateral to SCN in this species although they were not observed as far from the SCN as was seen in the three photoperiodic species. Inspection of the region surrounding the SON also failed to reveal bands of fibers passing dorsal to this nucleus. Intraocular injections of larger amounts of HRP in this species also resulted in cells within the PVN and SCN that appeared to be labeled with reaction product (Figure 15).

CONTROL CASES

Bilateral optic nerve transection. In the several cases (see Table 1) where the optic nerve was transected bilaterally prior to injection of HRP no reaction product was observed in any of the locations seen to contain reaction product following injections in intact animals.

Intravenous Injections. Injections of HRP directly into the systemic circulation followed by survival periods of at least 4 hr resulted in reaction product labeled cells in PVN, SON, a few labelled cells immediately adjacent to the third ventricle, and a small number of cells dorsal and lateral to SCN. The maximal amount of reaction product was

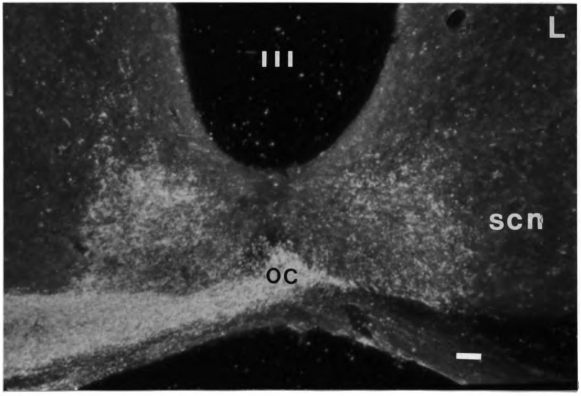

FIGURE 14. Asymmetrical RHT input to the SCN of a house mouse. The contralateral SCN contains a higher density of retinal input. Unilateral injection of HRP into the left eye. Coronal section from the caudal 1/2 of the SCN. (Dark-field illumination; Bar=50 μm).

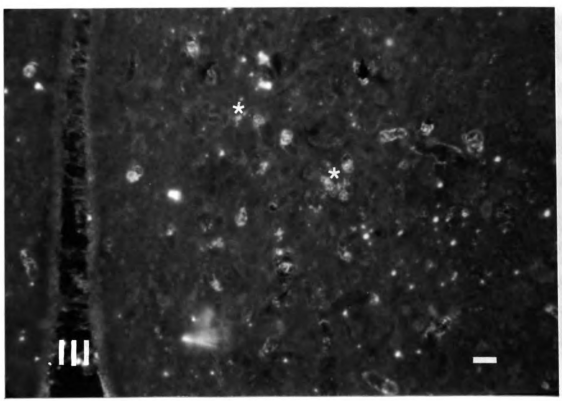

FIGURE 15. Cells and processes (asterisks) filled with HRP reaction product following bilateral intraocular injection of higher concentration and volume of HRP in a house mouse. (Coronal section; Darkfield illumination; Bar=20 μm).

observed following 8 hr survival in all 3 species. There was no reduction in concentration of reaction product observed after 24 hr survival. Cells observed in the PVN and SON appeared to be magnocellular (approximately 25 µm-35 µm; see Figure 16). Some of these cells in PVN exhibited processes that extended into the region between PVN and SCN. Processes of labelled SON cells extended dorsally and dorso-laterally. Cells located between PVN and SCN also occasionally displayed processes that approached SCN and PVN (Figure 17).

The most prominent difference between the photoperiodic deer and white-footed mice compared to non-photoperiodic house mice appeared to be the number and location of magnocellular units found dorsal and lateral to SCN. In the photoperiodic species a number of labelled cells were found quite close to the dorsal and lateral borders of the SCN. In some cases these cells were observed within the boundaries of the SCN as defined using cresyl-stained material. In contrast few cells were located adjacent to the dorsal and lateral edges of the nucleus in house mice. As for the lateral hypothalamus, a moderate number of cells were seen to contain reaction product in deer and white-footed mice where-as, again, fewer cells were seen in the house mouse.

Figure 16. Photomicrograph of magnocellular neurons within PVN (double arrowheads; upper panel) and SON (asterisks; lower panel) labelled following intravenous injection of HRP in a white-footed mouse. (Coronal section; Darkfield illumination; Bar=50 μm).

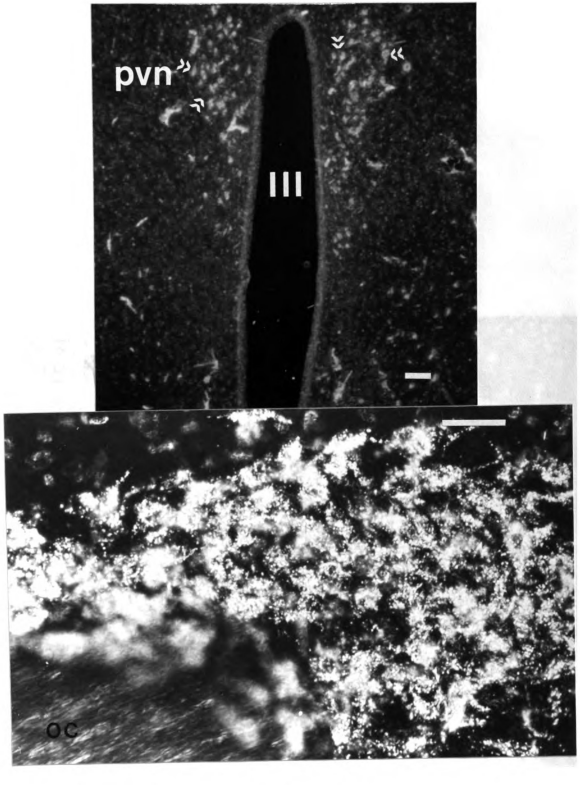

FIGURE 16.

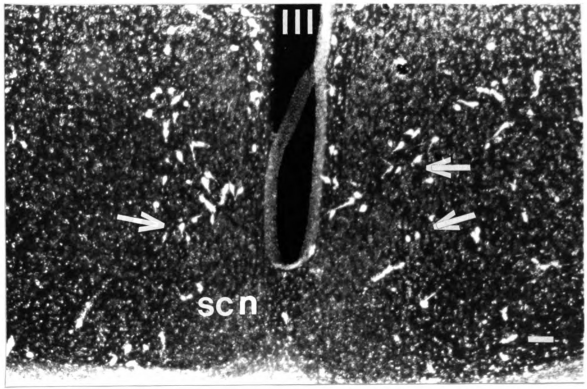

FIGURE 17. Magnocellular neurons and processes (arrows) located in/near the SCN of a white-footed mouse labelled by HRP that was injected into the general circulation. (Coronal section; Darkfield illumination; Bar=100 μm).

DISCUSSION

The anatomical substrate for a circadian clock has been shown to be the suprachiasmatic nuclei of the hypothalamus (Moore & Klein, 1974; Moore, 1978; Nishino, Koizumi & Brooks, 1976). Ablation of the SCN or isolation of the nuclei from the remainder of the brain results in a loss of rhythmicity in other areas of the brain and body (Inouye & Kawamura, 1982; Klein & Moore, 1979; Moore & Eichler, 1972; Rusak, 1977; Stephan & Zucker, 1972; Stetson & Watson-Whitmyer, 1976). Photoperiodic responses observed in certain species of mammals also are dependent upon SCN mediation (Rusak & Morin, 1976; Stetson & Whatson-Whitmyer, 1976). The photoperiodic response of seasonal regression and recrudescence of gonads is disrupted by lesions of the SCN. Neural activity of the SCN is modulated by environmental light impinging upon the retina (Takahashi & Zatz, 1982; Richter, 1965). The signal is transduced by the retina to a neural signal that is transmitted to the SCN via RHT fibers.

As has been previously described (Eichler & Moore, 1974; Hendrickson, Wagoner & Cowen, 1972; Moore, 1973; Moore & Lenn, 1972; Pickard, 1982; Pickard & Silverman, 1981; Printz & Hall 1974), the RHT and its pattern of termination within the SCN of golden hamsters has unique features when

compared to other mammalian species. Compared to RHT input by either eye in the rat, the observed concentration of RHT input to the SCN of the golden hamster is more symmetrical following unilateral intraocular injection of HRP. The ipsilateral SCN of the golden hamster contained a higher amount of input whenever differences in concentration were discerned. In the present study, the pattern of RHT input to the SCN of Turkish hamsters has been shown to be similar in appearance to that of the golden hamster after a unilateral intraocular HRP injection. The pattern of input is relatively equal bilaterally through the rostro-caudal extent of the SCN in both species of hamster. These two species of hamster are unique with respect to symmetry of retinal input to the hypothalamic SCN when compared to other non-photoperiodic species of mammals examined previously (Moore, 1973). Hamsters are also different when compared to photoperiodic ferrets in regard to symmetry of RHT input to the SCN (Thorpe, 1975).

Interpretation of the results of the present study in the light of the techniques utilized previously must be tentative. Regarding the golden hamster, a similar HRP-TMB technique was used to examine RHT input to the SCN (Pickard & Silverman,1981). Therefore, comparisons of the RHT input in the golden hamster to the pattern of input in the Turkish hamster are reasonable. However, to compare these two species of hamster to any other species requires consideration of the methods formerly utilized to visualize

the RHT. The earliest examinations (Moore, 1973; Moore & Eichler, 1972) of retinal input to the SCN were based upon autoradiography, using isotopes attached to amino acids. As is the case of HRP, amino acids were injected intraocularly. The autoradiographic technique was an improvement over nerve lesioning and silver staining (Cowan, Gottlieb, Hendrickson, Price & Woolsey, 1972). However, the sensitivity of autoradiography is less than that of methods based upon HRP. Even early HRP techniques still in use are not as capable of detecting small nerve processes or revealing small numbers of fibers as HRP-TMB (Mesulam & Rosene, 1979). Therefore, the results of studies utilizing methods of tracing nerve fibers other than HRP-TMB to reveal the RHT probably do not reflect the fullest extent of RHT input to the SCN.

In their their published report on of the RHT of the golden hamster using a HRP-TMB technique, Pickard and Silverman (1981) also reported asymmetrical RHT input to the SCN of two rats which were included in the experiment. Although for the rat they found a pattern of RHT input to the SCN similar to the pattern described by others using less sensitive techniques, the HRP-TMB technique has some inherent variability associated with it (Rosene & Mesulam, 1978; Mesulam et al., 1980). Thus, tracing RHT input to the SCN of rats using only two cases does not seem to be a rigorous examination of this species.

In the present study the house mouse was examined using the sensitive HRP-TMB reaction procedure and the pattern of termination within the SCN by RHT was seen to resemble that of the rat rather than that of the photoperiodic hamsters. Although, like the rat, the SCN of house mice contralateral to the injected eye reliably displayed higher concentrations of RHT input, both nuclei exhibited levels of retinal input higher than has been shown in the rat. The finding of asymmetrical RHT input in house mice is contrary to the citation made by Pickard and Silverman (1981) to argue that the concentration of input was relatively symmetrical bilaterally in the SCN of house mice. While they claim that the earlier report by Drager (1974) demonstrates nearly equal amounts of autoradiographic exposure of emulsion over the two nuclei there is little evidence found in Drager's work to support this observation. The presence of the apparent retinal input in the SCN or the region of the SCN was noted by Drager but thorough examination of the region of the brain including the SCN was not undertaken. Further, the technique utilized was not as sensitive as the HRP-TMB reaction and the possibility of trans-synaptic transport of the tracer chemical is significant. Since amino acids taken up by the cells would tend to spread throughout the cell and mask over the discrete terminals of retinal fibers. The masking would give the SCN the appearence of uniform density of labelling, especially at low magnification viewing of the tissue.

Examination of only the Turkish hamster and the house mouse supports the hypothesis that more symmetrical retinal input to the SCN is an anatomical feature of photoperiodic species. However, beside the various species of hamsters, other mammals have been shown to be photoperiodic (Clarke & Kennedy, 1967; Farrar & Clarke, 1976; Herbert, 1969; Thorpe & Herbert, 1976). To test the "symmetry of RHT input" hypothesis further, deer and white-footed mice were included in the present study. Examination of the photoperiodicity in deer and white-footed mice has revealed that many individuals of these 2 species are photoperiodic (Dark, Johnston, Healy & Zucker, 1983; Lynch, 1973). Thus, the hypothesis predicted that the RHT would terminate in a symmetrical pattern in the SCN of these photoperiodic species of mice. However, the present data appear to indicate that deer and white-footed mice were similar to non-photoperiodic house mice and rats rather than to the photoperiodic hamsters. In the two photoperiodic species of mice, the SCN contralateral with respect to the HRP injected eye displayed a higher amount of retinal input. This higher concentration of input was most evident in the caudal 1/2 of the nuclei of both species. From the data obtained in the present study it is clear that symmetry of RHT termination in the SCN is not a universal feature of photoperiodic species. Instead, among rodents, symmetry of RHT input to the SCN appears to be a hamster-specific feature. In addition, ferrets display a pattern of retinal input similar to that of

non-photoperiodic rodents (Thorpe, 1975).

In the animals examined in the present study, locations within the nuclei where RHT input terminated were seen to contrast with most of the data from previous reports (Moore, 1973; Moore & Eichler, 1972; Thorpe, 1975). The rat has been reported to receive a large portion of RHT input in the ventral area of each SCN with minimal RHT input to dorsal areas of either nucleus when autoradiographic techniques are employed (Moore & Lenn, 1972). Pickard and Silverman (1981) replicated this observation in two cases applying the HRP-TMB technique. In Turkish hamsters a pattern of RHT input similar to the pattern seen in the golden hamsters was observed. There were low concentrations of RHT input to the medial and dorsal SCN while the ventral to dorso-lateral areas of the SCN had relatively higher concentrations of input. In the case of golden hamsters there appears to be high concentrations of retinal input to the ventral, lateral and dorso-lateral regions of the SCN bilaterally (Pickard & Silverman, 1981). Similarly, deer and white-footed mice tended to display highest concentrations of retinal input to dorso-lateral, lateral and ventral SCN rather than restricted to the ventral 1/3 of the SCN. House mice differed from rats for the same reason; that is, a larger area of the SCN contained retinal input in house mice than was reported previously for the rat. Ventral SCN did contain high concentrations of retinal input but rather than being confined to the ventral 1/3 to 1/2 of the nucleus as has been shown in

the rat, the house mouse was found to have a region of high concentration of input that extended through the ventral 2/3 of the suprachiasmatic nuclei. All species examined were similar in that the dorsal and dorso-medial SCN contained the lowest amounts of RHT input. The results of this study are in contrast to previous examinations of mammalian species as RHT termination has been reported to be restricted to ventral SCN. However, as was discussed above, studies based upon techniques other than HRP-TMB may be less sensitive in detecting the small number of fibers present in the RHT, especially those fibers located in dorsal SCN. Until HRP-TMB can be applied in reexamining these species, comparisons are at best tentative.

Since the identification of the RHT there has been an interest in identifing sites in which the RHT terminates other than the SCN (Hendrickson, Wagoner & Cowan, 1972; Moore & Lenn, 1972). The SON and PVN are regions of the hypothalamus involved in the release of hormones significant for reproductive functions and, at least the PVN, is directly involved in light-mediated release of pineal melatonin (Klein, Smoot, Weller, Higa, Markey, Creed & Jacobowitz, 1983). A direct connection to either of these paired nuclei from or to the retina could be part of a mechanism for controlling the neural activity responsible for generating seasonal and circadian rhythms in endocrine functions. For example, one early examination of circadian rhythms in rats found a change in appearance of SON cells stained with

cresyl violet. The change in cells had a circadian component that was correlated with changes in water intake and urine output (Glantz, 1967).

Single fibers of the RHT have been traced as the processes enter the SCN (Murakami, Miller & Fuller, 1984). Some of these fibers have been shown to pass through the SCN and extend dorso-laterally or dorso-medially beyond the boundaries of the SCN. In the present study evidence of RHT fibers located outside the SCN was found. The features of extra-SCN input by the RHT were most prevalent in the photoperiodic species examined here. Intraocular injections of HRP in many Turkish hamsters, deer and white-footed mice resulted in fibers that were traced in the periventricular region dorsally to the area just ventral to the boundary of PVN. Additional fibers were found in the dorso-lateral hypothalamus, and when SON was examined fibers could be located dorsal to the SON boundary. Occasionally, evidence for fibers within SON and PVN were seen. Other than the diffuse projections of the RHT fibers into dorso-lateral hypothalamus there was no evidence for termination of retinal processes in other discrete nuclei within the hypothalamus. The house mouse displayed similar, though attenuated, features. Thus, RHT fibers were occasionally seen beyond the boundaries of the SCN and the optic tract of house mice. However, the RHT fibers could not be traced as far laterally and dorsally from the SCN as could be accomplished in the photoperiodic species.

In cases of deer mice, additional RHT fibers could be traced beyond the boundaries of the SCN following intraocular injections of HRP administered in higher volume and concentration. On the other hand, similar injections did not label additional fibers in house mice. After these injections of HRP, cells of the PVN and SON were labelled in deer mice. Though few fibers were traced beyond the boundary of the SCN in house mice, cells of PVN and SON also were labelled in this species. The presence in house and deer mice of labelled cells in the hypothalamic nuclei other than SCN suggests that the retinae may receive neural input from PVN and SON. This has been reported in dogs (Terubayashi, Fujisawa, Itoi & Ibata, 1983) and rabbits (Brandenburg, Bobbert & Eggelmeyer, 1981). Further, the data suggest that the RHT fibers seen beyond SCN could be processes of PVN and SON cells rather than those of retinal ganglion cells. Alternatively, the fibers of the RHT may have released HRP that in turn has been taken up by the cells of the PVN and SON, although this is not likely given the survival times employed in this study.

While the current study was unable to demonstrate conclusively the presence of retino-hypothalamic tract termination in areas of the hypothalamus other than the SCN, the possiblity must remain and it is suggested by other published reports (Fuller, Murakami & Miller, 1984; Kita & Oomura, 1982; Mai, 1979; Stephan, Berkley & Moss, 1981). The observations by Glantz (1967) that SON cells change their

appearance in synchrony with the L:D cycle suggests that light modulates the activity of these neurons. The source of input to SON may be direct via the RHT or by way of connections between the SCN and the SON (Mai, 1979; Stephan et al., 1981). Stephan and co-workers (1981) noted lightly labelled fibers dorsal to SON after intraocular injections of tritiated leucine but heavy labelling over SON after intraocular injections of labelled fucose and proline. These last two chemicals are known to be transported transsynaptically, thus suggesting SCN input to this region of the hypothalamus. The fibers seen over SON in the current study seem to be direct retinal projections and not second order fibers originating in the SCN. Regardless of amount of HRP injected intraocularly, a few labelled fibers were observed in SON.

Light mediated responses by the PVN are well documented. However, no evidence has been presented by any investigator to support the suggestion that PVN receives direct RHT input. Pickard and Silverman (1981) reported RHT fibers dorsal to SCN in the periventricular region that approached PVN and spread dorso-laterally in the hypothalamus. The specific locations of terminating fibers could not be determined. However, serial reconstructions of retinal axons by Murakami and co-workers (1984) suggest that RHT fibers passing through SCN terminate in the region dorsal to SCN but ventral to PVN. Dorsal projections beyond SCN were found in the present study but could not be traced into PVN. Possibly

RHT fibers do in fact terminate on cells outside traditional boundaries of known hypothalamic nuclei. Definite proof of a connection between the retinae and SON or PVN may require manipulations based upon electrophysiological recording in the paired nuclei. Single unit recording might be made while stimulating the optic nerve in brain slice preparations. Use of tissue slice preparations *in vitro* removes the potential confounding effect of blood born chemicals. Application of HRP to optic nerve and electron microscopic examination of the tissue for retinal fiber terminals may also illucidate RHT connections to PVN and SON.

The observation of magnocellular units within the PVN and SON labelled following intraocular injections suggests a possible confounding factor in the interpretation of data presented in this study and those data of previous studies that used a similar HRP technique for tracing the RHT to its areas of termination. If PVN and SON cells are labelled after intraocular injections of HRP both due to their own processes terminating within the retina and terminating upon nearby fenestrated blood vessels in the hypothatlamus, then a protocol for separating these two sources of observed reaction product becomes necessary. Several control cases in which the optic nerves were severed and then the eyes were injected with HRP two weeks later revealed no cells or fibers containing reaction product. Yet, the possibility remains that the blood supply to the eyes had been compromised after the nerve transections. If, however, there

remained an appropriate blood supply then the magnocellular units labelled after intraocular injections represent a site of termination for RHT fibers or alternatively, cells that project to the retina.

Intravenous injections of HRP in the rat and house mouse have shown that cells within the PVN and SON can take up and transport the enzyme from the median eminence and posterior pituitary (Armstrong & Hatton, 1980; Broadwell & Brightman, 1976). In this study, HRP-TMB reaction product was observed in processes that could be traced to nearby cells of the PVN and SON following intravenous injections in house and deer mice. The animals that received HRP intravenously were compared to cases of the same species that had been injected intraocularly. Data from intravenously injected animals suggest that the cells and processes labelled following eye injection might be interpreted as the result of RHT transport when in fact such staining was the result of endocytosis of HRP after intravenous transport of the enzyme. Thus, in cases with eye injections, the origin of the HRP that is finally located within the PVN and SON cells may be either the result of anterograde or retrograde neural transport; or could represent HRP which escapes the eyeball during or following the injection and reaches the brain via the general circulation. An alternate possiblity is the transsynaptic transport of the HRP at RHT terminals in or near the SCN. Colman, Scalia & Cabrales (1976) demonstrated HRP within phagocytes and endothelial cells following

intraocular injections in mice permitted to survive 18 hours. Considering the distance that the HRP is transported in the deer and white-footed mice it is a reasonable idea that the HRP has reached the RHT terminals within the SCN following 6-8 hours. The amount of time remaining until the animals were sacrificed would seem to be sufficient for some of the HRP to be released from the terminals.

The presence of magnocellular components outside of PVN and SON represent a new finding in deer and white-footed mice. Interestingly, the number of cells located near the SCN of house mice was not as high as was found in the deer and white-footed mice. Also, cells which were identified in house mice through out the hypothalamus and outside PVN and SON were fewer in number. Although a Nucleus Circularis has been reported previously in rats (Peterson, 1966) and mice (Broadwell & Brightman, 1976) the magnocellular neurons identified adjacent to the SCN in deer and white-footed mice do not appear to correspond to the suggested locations of N. curcularis in rodents. The presence of magnocellular units labelled after intravenous injections suggest two interesting ideas. First, as stated above, these cells present a possible confounding factor to the interpretation of RHT input to the hypothalamus as do the labelled cells of PVN and SON. Magnocellular units and their associated processes so close to the boundaries of SCN could easily be miss identified as representing RHT fibers or terminals. Misinterpretation would be more likely in the case of

lightly stained cells as would probably be the case if only a slight amount of HRP was transported by the blood. The relative absence of similarly located cells in house mice might explain the apparently reduced level of RHT input to the rest of the hypothalamus in this species.

Second, these cells adjacent to the SCN may mediate communication between the SCN and the rest of the body. Neural activity within the SCN may cause the release of hormones into the blood by these magnocellular units. Many of the magnocellular neurones of the hypothalamus have been found to contain hormones known to be released in a circadian cycle into the blood and CSF (Schwartz, Coleman & Reppert, 1983; Silverman & Zimmerman, 1983). The positioning of cells, that may be intimately involved in endocrine functions, adjacent to the SCN could provide a mechanism for the circadian control of hormone release. On the other hand, the magnocellular neurons are adjacent to capillaries which may be fenestrated and, thus, are capable of taking up blood born chemicals that modulate neural function.

In the present study symmetry, or at least reduced asymmetry, was not observed in deer mice or white-footed mice. Thus, the differences in retinal input to each SCN, (i.e., symmetry vs asymmetry) may be a genus specific feature restricted to hamsters and not a general feature of photoperiodic rodents. Examination of the djungarian hamster may provide evidence to support this hypothesis. The functional significance of symmetry of RHT input remains

unknown. In the light of the anatomical feature of symmetry some interesting behavioral data may be explainable. Stephan, Donaldson and Gellert (1982) examined the entrainment of circadian rhythms in rodents who were intact or had an unilateral enucleation. These results suggest that an animal's ability to reentrain a rhythm following a phase shift is dependent upon the symmetrical distribution of RHT fibers that reach the SCN. Thus, in hamsters unilateral enucleation does not affect reentrainment after a phase shift, but in rats unilateral enucleation delays reentrainment.

A similarity in RHT input to the SCN might suggest a similarity in effect of light input upon SCN activity in the golden and Turkish hamsters. It seems important to identify structural differences in the brains of photoperiodic species which set them apart from the brains of non-photoperiodic animals. Differentiation based upon anatomical features may suggests avenues of exploration for locating key differences in cell morphology, neurochemistry and physiology. The presence of the magnocellular neurons and their density near the SCN of deer and white-footed mice coupled with the relative absence of similar cells in the hypothalamus of house mice suggests that these cells may represent an anatomical feature of photoperiodic species. The SCN are an integral part of the mechanism which controls seasonal cycles in response to changes in photoperiod. The photoperiodic response involves melatonin induced changes in

the feedback mechanism by which gonadotrophin release is modulated (Sisk & Turek, 1982). Thus, if the SCN is to modulate responses to circulating levels of hormones, a mechanism for sampling blood born chemicals may be required. Cells of the SCN or near the SCN appear incapable of taking up chemicals from the general circulation in rats (Miller, Lauber & Fuller, 1984) and mice (present data). On the other hand, the magnocellular units adjacent to the SCN seen in deer and white-footed mice could provide a mechanism for chemical feedback to the SCN. Further testing of this idea is, of course, required. Not all adult deer mice (Dark, et al., 1983; Whitsett & Lawton, 1982) and white-footed mice are photoperiodic (Gram, Heath, Wichman & Lynch, 1982; Lynch & Wichman, 1981). Once responsiveness is established among individual animals the anatomy of the hypothalamus could be correlated with emphasis on number and location of magnocellular components in the region of SCN. In addition, golden and Turkish hamsters should be examined in the light of the data from deer and white-footed mice. These two species of hamsters respond differently to pinealectomy (Goldman & Darrow, 1983) and so may display differential anatomical features with respect to magnocellular components near the SCN.

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