The Hypothalamic Suprachiasmatic Nucleus of Rat: Intrinsinc Anatomy

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ABSTRACT The internal structure of the suprachiasmatic nucleus (SCN) was studied qualitatively and quantitatively in several hundred rat brains with a variety of methods including several types of Golgi impregnations, Nissl stains, horseradish peroxidase application, and electron microscopy. One suprachiasmatic nucleus has a volume of about 0.068 mm³ and contains close to 8,000 neurons. Dorsomedially, cells tend to be smaller and more tightly packed than ventrolaterally; significantly more somatic appositions occur in the dorsomedial SCN than in other parts of the nucleus. Two neurons with an extended region of somatic apposition may have no intercellular specializations, or they may be held together with various attachment plaques. Chains of neurons with long regions of somato-somatic apposition are found in the dorsomedial SCN with Nissl and silver stains, and with EM. The length of these chains is generally oriented in an antero-posterior direction. Interspersed with the neurons are astroglia. Astroglia studied with Golgi impregnations, Cajal's gold sublimate stain, and EM in SCN may have a rich cytoplasm falling in between the organelle-rich cytoplasm of some large neurons and the organelle-poor cytoplasm of some of the smaller SCN neurons. Nuclei of SCN glia and neurons are often invaginated and multiple nucleoli are a prominent feature of a large number of SCN neurons. With Golgi impregnations a number of relatively simple dendritic arbors exist. These include the simple bipolar cell, curly bipolar cell, radial neuron, monopolar neuron, and spino-spinal cell. At the borders of the nucleus some dendrites may travel into the adjacent anterior hypothalamus; similarly, dendrites from cells outside SCN may enter into the nucleus boundaries. Compared with the rest of the hypothalamus, axons in SCN stain very poorly with conventional histological methods including Luxol blue, Bodian, Sevier-Munger, Loyez, and Golgi, in part because of the fine caliber of the fibers. Golgi impregnated and silver-stained axon fascicles composed primarily of unmyelinated axons may divide up within the nucleus or may pass through without maintaining local collaterals. Several different types of Golgi-impregnated axonal arborizations terminate within SCN. Some have an extensive number of boutons ending on SCN somata, dendrites, and dendritic appendages. Other single axons pass through SCN without leaving any collaterals, or terminals in the nucleus. The majority of Golgi-impregnated axons arising from SCN neurons maintain locally terminating collaterals, with boutons en passant and termineaux; these axons originate with equal frequency from the perikaryon or from a proximal dendrite. Golgi, horseradish peroxidase, and silver staining methods reveal axons connecting left and right SCN.

The SCN is a complex nucleus with recognizable subdivisions that contain neurons with several types of relatively simple dendritic arbors. Within any area of SCN, ultrastructural differences can be found between neighboring cells, suggesting a heterogenous population of neurons. Combining the results of the present study with previously reported data, neurons of the SCN have a large number of
possibilities for intercellular communication between cells within the nucleus. These include a few presynaptic dendrites and somata, frequent local circuit axons, and possibly ephaptic interaction between closely apposed cells.

The hypothalamic suprachiasmatic nucleus has been the subject of recent attention for its role in the mediation of behavioral and endocrine rhythmical events. Stephan and Zucker ('72) and Moore and Eichler ('72) showed that lesions in the area of the SCN abolished circadian rhythms of drinking and locomotor activity as well as corticosterone. Other investigators have found that lesions in the SCN area eliminated diurnal and circadian variations of pineal serotonin N-acetyltransferase (Moore and Klein, '74), heart rate (Saleh and Winget, '77), and sleep (Ibuka and Kawamura, '75; Cointet et al., '75). A recent fine-grained anatomical analysis substantiated hypotheses of the earlier workers that the SCN is a critical group of cells involved in certain circadian rhythms (van den Pol and Powley, '79). Additional work has suggested that SCN may play a crucial role in the estrous rhythm of rats (Brown-Grant and Raisman, '77; Raisman and Brown-Grant, '77; Critchlow, '63; Stetson and Watson-Whitmyre, '76), the regulation of the luteinizing hormone surge (Coen and MacKinnon, '76, '77), and memory processes (Wansley and Holloway, '75; Buijs et al., '78).

Anatomically, the SCN receives two visually related projections, one directly from the retina (Hendrickson et al., '72; Moore and Lenn, '72) and another from the ventral lateral geniculate (Ribak and Peters, '75; Swanson et al., '74; Groos and Mason, '78). Other afferent projections arise from the raphe (Aghajanian et al., '69; Be, '65) and subiculum (Meibach and Siegel, '77). SCN efferent projections are found posterior to SCN in the mediobasal hypothalamus (Makara and Hodacs, '75; Swanson and Cowan, '75). With immunohistochemical methods, SCN fibers containing antidiuretic hormone and neurophysin have been reported to project to or through a large number of hypothalamic and nonhypothalamic loci including the medial and lateral septum, medial dorsal thalamus, lateral habenula, nucleus of the diagonal tract of Broca, interpeduncular nucleus, central grey of the mesencephalon, dorsal motor nucleus of the vagus, posterior hypothalamus, preoptic periventricular nucleus, and organum vasculosum of the lamina terminalis (Sofroniew and Weindl, '78; Buijs et al., '78; Buijs, '78). Whether SCN axons make synaptic contact in these regions is still an unresolved question. As fibers from nearby supraoptic, paraventricular, and accessory magnocellular neurons contain ADH and neurophysin, and may mingle with SCN axons, additional verification of immunocytochemically detected SCN projections will be valuable. Ultrastructural studies on the synaptology of SCN have been reported by Güldner ('76, '78), Lenn et al. ('77), and Suburo and Pellgrino de Iraldi ('69).

The central focus of the present study is on the intrinsic anatomy of the SCN. Within this general framework, I have examined regional variations in cell size and packing density within the nucleus, types of dendritic arborizations, neuropil, and morphological substrates for intercellular communication within the nucleus with a number of converging anatomical methods at the light and electron microscope level. This is the first in a series of papers concentrating on the intrinsic organization of medial hypothalamic nuclei. A preliminary description of some of these data has been reported in abstract form (van den Pol, '78).

**METHOD**

**Rats**

The suprachiasmatic nuclei from over 300 rats were studied. To reduce animal-to-animal variance, highly inbred Fischer albinos (Microbiologica, Walkerville, Maryland) were used. Albino rats of undetermined genetic background were also used for some Golgi impregnations. Except for the Golgi work, all animals were young adult males 55–70 days of age. Both males and females of various ages were used in the Golgi studies. All adult animals were perfused intracardially using a gravity feed perfusion apparatus. Young rats were perfused with a 22-gauge, non-beveled needle on a 50-ml syringe.

**Nissl**

To count the number of cells in SCN, formalin-fixed brains of three males were embedded simultaneously in paraffin with a Lipshaw Automatic Tissue Embedder. Serial 12-μm horizontal sections were cut through the hypothalamus. The Klüver-Barrera stain for Nissl substance and myelinated fibers was used.

To examine subpopulations of cells within the SCN, the brains of four Fischer male rats were perfused with 10% phosphate buffered
formalin and the SCN area was block-stained in osmium and embedded in Epon. Five 1-μ horizontal sections stained with toluidine blue were saved at 50-μ intervals through the ventrodorsal extent of the nucleus. One of the four brains was discarded from the analysis as its plane of orientation was not parallel to the other three. A photomontage of the entire SCN at each level was made with a print linear magnification of 4,000 ×. On the basis of variations in cell density in the different regions of SCN, cross-sectional areas of cells were measured with a Numonics Digital Planimeter.

Table 1 lists all of the histological methods used in the present study of SCN.

**Golgi impregnations**

A total of 231 animals were used for Golgi impregnation. Thirty-five of these were used for Golgi-EM as described below. The rapid Golgi as described by Valverde ('70) was used on 24 rats. This procedure was especially useful in impregnating long axons, although often cells were not stained. Six rats were treated according to the procedure outlined by Somogyi ('78). Eight rats were treated according to the method of Szentagothai ('63). Variations of the Stensaas and Stensaas ('68) modification of del Rio Hortega's Golgi proved useful, especially for impregnating glial elements and neurons with short axons. A large number of brains were impregnated with unpublished combinations or variations of Golgi methods. Single, double, and triple impregnations were tried; single impregnations proved most useful. Incubation times in the initial holding solution varied from three hours to three weeks. Transcardial perfusion was used in most animals, although some well-impregnated cells were obtained with immersion fixation. Of the 231 rats, 205 were of the inbred Fischer strain, 12 were of nonidentified strains obtained in Budapest, and 14 were Sprague-Dawleys. Ages ranged from 3 days to 75 days. In general, older animals yielded fewer impregnated cells; this may have been due to the increased myelinization in the older animals. Golgi methods were expectedly variable in their success. In some brains only axons or glia were impregnated. In others from 1 to 500 neurons were filled with silver chromate. In general, cells were reddish-brown or black. The lighter impregnated structures seemed to show better detail in accordance with previous findings (Palay and Chan-Palay, '72). In most cases brains were infiltrated with celloidin and cut with a sliding microtome in serial coronal, sagittal, or horizontal sections. Thickness varied from 64 micra to 175 micra. Some impregnated brains were also cut by hand with a razor blade or with a Sorvall TC2 Tissue Sectioner. Analysis of local topography including the third ventricle, tractus infundibularis, optic chiasm, and supraoptic commissure facilitated positive identification of SCN cells. To further verify localization, some impregnated brains were embedded in Epon and cut in one-micron sections on a Reichert OM U3 ultramicrotome with glass knives and counterstained with osmium tetroxide and toluidine blue.
Golgi-EM

Several methods of Golgi-EM were used. The Stensaas and Stensaas ('68) Golgi preparation was used with eight specimens to impregnate SCN neurons lightly. The best Golgi impregnations with this method were obtained when the brains were initially immersed in the fixative with an acidic pH 4.4. The low pH was not conducive to a well-preserved ultrastructure, and consequently little of this material was useful for ultrastructural analysis. Golgi impregnations were also obtained in 35 rats with the methods of Fairen et al. ('77), Somogyi ('78), and Blackstad ('65). Slabs were cut either by hand with a razor blade or with a Sorvall TC2 tissue sectioner. From the same block, some slabs were gold-toned according to the procedure described by Fairen et al. ('77) and others were left impregnated with silver chromate. Thick Golgi sections were block-stained with ethanolic uranyl acetate. After embedding in plastic, thin sections from gold-toned specimens were cut with a Reichert OM-U3 ultramicrotome into a water trough, while sections from silver chromate material were cut into a solution of saturated silver chromate (Blackstad, '65; Somogyi, '78). The present paper will draw from the EM-Golgi material primarily for light microscopy; a later paper will concentrate on EM.

Ultrastructural cytology

With one exception noted below, animals used for ultrastructural examination were perfused transcardially with one of several fixatives. In some cases fixative perfusion was preceded by perfusion with 0.9% saline either with or without heparin. Various concentrations of glutaraldehyde and formaldehyde were tried with varying degrees of success. Either phosphate, cacodylate, or arsenate was used as a buffer; pH was held at 7.3 ± 0.1. The fixative combination that resulted most reliably in good fixation was 3% glutaraldehyde and 2.5% paraformaldehyde in a cacodylate buffer. The brain was removed from the perfused animal within ten minutes to two hours after perfusion was completed, and the SCN area was dissected out and immersed in fixative for one hour. The SCN was then cut out of the block and left in fixative for several hours, and then either put in a buffer or osmicated, dehydrated, and embedded in plastic.

A number of en bloc stains and mordants were used: Some blocks were stained with uranyl acetate immediately after osmication, or after a buffer rinse. Other blocks were treated with the low molecular weight galloyl-glucose mordant tannic acid after osmication. As tannic acids vary considerably from company to company, the tannic acid produced by Mallinckrodt, reported to be optimal for the use as described herein (Simionescu and Simionescu, '76), was used. Two blocks were fixed directly in osmium without the aldehyde perfusion. Lanthanum nitrate was used in two blocks according to the procedure outlined by Revel and Karnovsky ('67) for showing the detail of gap junctions: Rats were perfused with 0.1 M cacodylate buffered 3% glutaraldehyde and 2.5% paraformaldehyde, the SCN area was removed and immersed in the same cacodylate buffer with 1% lanthanum nitrate for 90 minutes, and the block was then immersed in a 1% buffered osmium 1% lanthanum solution for 75 minutes and subsequently embedded in plastic.

The SCN area was dehydrated in ascending ethanol series and embedded in Epon, Spurr’s media, or Durcupan. Silver sections were cut on a Reichert OM U3 ultramicrotome. A 1-µ-thick section stained with toluidine blue was saved immediately prior to each set of thin sections. For high magnification examination 300 or 400 mesh grids were used, while for lower magnifications formvar-coated slot grids were used. The slot grids were also used for serial sections. In some cases Maxtaform Finder grids were used; these map grids are composed of miniature letters which facilitate relocation of specific structures. Map grids were also used to compare the same neural locus before and after certain staining procedures.

Thin sections were examined without any staining from blocks that had been osmicated or immersed in lanthanum. Uranyl acetate and lead citrate or lead citrate alone was used to stain some sections from blocks that received aldehyde fixation and osmication. Lead citrate was used without uranyl acetate for the blocks that had been immersed in the tannic acid mordant.

Horseradish peroxidase application

HRP was applied either with pressure injections from a Hamilton microsyringe or with iontophoresis. Both in vivo and in vitro preparations were used. Microiontophoresis in vivo gave the clearest picture. A constant current source producing 2 microamps was used; current was applied for 0.5 seconds, and off for 0.5 seconds for a total duration of 3–15 minutes. A glass pipette with a tip diameter of 8–50 µ was filled with 4% HRP (Sigma Type VI, or Worthington). The tip of the pipette or needle
was stereotaxically placed in or near the SCN. The coordinates for the lateral part of SCN were 9.1 mm down from the skull surface, 0.5 mm lateral, and 1.1 mm posterior to bregma; the rat's head was held by a mouth-nose clamp in a homemade stereotaxic instrument with the dorsal surface of the skull maintained horizontally. Animals were sacrificed one hour to two days after HRP application. Fifty or 100 micra serial sections were reacted with diaminobenzidine or the Hanker-Yates reagent. Optimal coloration of local axons and dendrites was seen when animals were sacrificed three hours after HRP iontophoresis. In a few cases sections were counterstained with cresyl violet, but in most cases they were not since the cresyl violet tended to obscure some of the finer detail and contrast of HRP-filled structures.

The use of HRP allowed an independent check on the possibility that the Golgi method might be selectively staining certain cells within the SCN. The use of HRP is based on the working assumption that the enzyme not only will demonstrate cells with axon terminals in the area of HRP application but, more importantly in this case, will label a large number of cells with broken membranes. Two shortcomings of the HRP method for examination of local dendrites and axons are that it selectively labels damaged cells and that with HRP the detail is seldom as good as seen in a Golgi impregnation. Dendritic spines, for instance, were much more apparent with the Golgi method than with HRP.

Microscopy

Golgi and HRP material was examined under both light- and darkfield conditions. Thin axons often were more visible with darkfield illumination. For critical examination, thick sections were studied under long working distance Zeiss Neofluar 63× and 100× objectives with numerical apertures of 1.25 and 1.30, respectively. In Golgi preparations where details were obscured by other structures superimposed, the microscope slide was turned upside down for further examination. A Zeiss camera lucida drawing tube was used to make drawings. A Philips 201 electron microscope was used for ultrastructural work. Objective apertures of 20-μ or 50-μ diameter in combination with condenser apertures of 50- or 200-μ diameter were used, usually in the 40,000 to 80,000 volt range.

Unless otherwise indicated, magnifications in figure captions give the total width of each photomicrograph.

RESULTS

Anteroventrally SCN is bounded by the optic chiasm, and posterovertrally by the supraoptic commissure. The fibers of the supraoptic commissure are closely juxtaposed with those in the optic chiasm, and axons of the supraoptic commissure can be mistaken for chiasmal fibers. In horizontal sections tractus infundibularis is seen as it appears to leave the optic chiasm and pass ventral to the third ventricle (Fig. 1A). The third ventricle divides the paired suprachiasmatic nuclei in their dorsal aspect. A cell-free zone of about 60 μ separates the medial SCN from the wall of the third ventricle (Fig. 2F). One conspicuous trait of the SCN is the tight packing density of the small neurons and relative rarity of axons stained with conventional light microscopy techniques (Fig. 1A, B).

SCN size

From measurements made in ten brains, the SCN is about twice as long as it is wide. In ten paraffin-embedded brains the anterior–posterior length was 947 ± 56 (x ± SD) μ. These measurements were taken at the longest extent of SCN in serial horizontal sections. The width of the nucleus at its widest point was 424 ± 18 (x ± SD) μ. As these measurements were made in horizontal sections, the dorsoventral measurement could not be made directly. However, by measuring the ratio of the height of SCN to its width in ten coronally sectioned paraffin-embedded rat brains, a ratio of h:w of 0.92 ± 0.06 (x ± SD) was found. The medial SCN extends more dorsally than the lateral SCN and was used for these calculations. With a mean width of 424 μ the height of SCN in these brains could be calculated to be 390 ± 25 (SD) μ. A rough estimate of the volume of one suprachiasmatic nucleus can be found by multiplying h × w × l giving 157 × 10⁶ μ³ (0.16 mm³). To determine a more exact estimate of SCN volume, the area of SCN on serial horizontal sections from three paraffin-embedded brains was traced with a camera lucida drawing tube. In each brain SCN appeared on 24 or 25 serial sections. While the paraffin sections were cut with a rotary microtome set for 12-μ thickness, rehydration causes an increase in section size. By using the height of the nucleus of 390 μ and assuming that sections are equally distributed through this dorsoventral measurement, a corrected section thickness of 390 / 25, or 15.6 μ can be estimated. By multiplying the area of each section by its thickness and adding the volume of SCN in each section,
the total volume of one suprachiasmatic nucleus was calculated as $6.82 \times 10^7 \pm 8 \times 10^6 \mu^3$ (or $0.068 \pm 0.08 \text{mm}^3$). Both suprachiasmatic nuclei then would account for 0.136 cubic mm of rat brain.

**Cell density**

While all the cells of SCN are more tightly packed than the adjacent anterior hypothalamus, those in the dorsomedial part are even more tightly packed that those in other parts of the nucleus (Figs. 2, 3). Cells in the dorsolateral part of what is often considered the SCN are even more tightly packed than those in other parts of the nucleus. As cells in this part of the SCN have other characteristics that set them apart from more medial and ventral cells but are closely similar to cells in the adjoining anterior hypothalamus, one could make a case that these dorsolateral cells should be classified with the anterior hypothalamus rather than the SCN. Guldner ('76), in his thorough study on the synaptology of the SCN, recognized the difficulty in differentiating the dorsolateral SCN from the area outside the SCN and, to reduce problems of identification of true SCN cells, deleted this area from his morphological analysis. Similarly, the anterior SCN, especially laterally, tends to fade gradually into the surrounding hypothalamus. Cells here may be slightly larger and less packed than in other regions of SCN. Figure 3 shows the neuron density in representative squares 100 $\mu \text{on edge}$ throughout the SCN and in the immediately adjoining anterior hypothalamus. The outer boundaries of SCN in the dorsolateral and anterior SCN are difficult to define with exactitude given the smooth transition between SCN and the adjacent hypothalamus.

**Number of cells in the SCN**

Three paraffin-embedded brains were cut in 12 micra horizontal sections and stained with the Kluver-Barrera (53) stain for cell bodies and myelinated fibers. With the exception of cells that were obviously astrocytes or microglia as characterized by a small, densely staining cell body, all cells in the SCN were counted. The number of sections that contained SCN varied between 25 and 27. The number of cells counted in one suprachiasmatic nucleus from each of three brains was 10,794, 11,804, and 9,870, with a mean of 10,823. As all neurons in all serial sections through the SCN were counted, using presence of the nucleus as a criterion for counting, the mean of 10,823 is probably an overestimate as some cells might appear on two consecutive sections. To compensate for these split neurons a number of formulae have been developed. Assuming a real section thickness of 15.6 $\mu$, a nuclear diameter of 5 $\mu$, and a minimum nuclear criterion diameter of 2 $\mu$, the real number of neurons in SCN can be estimated by multiplying the counted number by 0.76 (Abercrombie, '46), 0.77 (Flanders, '44), or 0.77 (Konigsmark, '70). Further discussion of split neuron correction factors can be found in Konigsmark's paper. In a paraffin section it is difficult to distinguish

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Fig. 1. A. The suprachiasmatic nucleus from 12-μ horizontal section. The close packing density of its cells makes the SCN clearly distinct from the surrounding anterior hypothalamus. The top of the micrograph is anterior. The star denotes the anterior lateral SCN where the cells are larger and farther apart than in other parts of the nucleus. Krieg's ('32) tractus infundibularis bisects the left and right SCN. Silver-protein stain. 1.4 mm. B. Darkfield photo montage from silver stain for cells and fibers, horizontal section. A dramatic transition is found at the SCN lateral border: On the right in SCN only a few axons and many cells are seen, while on the left in the anterior hypothalamus fewer cells and many stained axons are found. Many axons lateral to SCN run in a rostro-caudal direction. With several silver stains for axons, SCN shows a significant lack of stained axons, in contrast to other hypothalamic nuclei. This may be due to the thinner diameter of SCN fibers, or due to some slight histochemical difference between SCN and non-SCN hypothalamic loci. 240 μ.
Fig. 2. A–E. These frontal sections show SCN from anterior (A) to posterior (E). Rostrally, SCN begins in a slight indentation in the optic chiasm. In B and C the depression in the optic chiasm is quite noticeable. The axons running ventral to SCN in E are primarily from the post-optic commissures. Fibers ventral to SCN in D are a combination of optic chiasm and post-optic commissures. SCN is largest in C and narrows down both rostrally and caudally. A–E, same magnification, 850 μ. F. Horizontal section through part of dorsol SCN. A cell-poor zone (CPZ) of about 60 micra is found between the wall of the third ventricle and the most medial cells of SCN. The few cells seen in the cell-poor zone are predominantly glia. EM analysis of the region reveals many axons. The density of SCN cells adjacent to the cell-poor zone (i.e., medial) is higher than in the area farther away from the ventricle. 480 μ.
SUPRACHIASMATIC NUCLEUS INTRINSIC ANATOMY

A

B

C

D

E

F

SCN

OC

3V

CPZ

3V

SCN
Fig. 3. This figure is derived from measurement of cell density in squares 100 micra on an edge at 55-μ intervals going from ventral SCN (bottom of figure, row A) to the anterior hypothalamic immediately dorsal to SCN (top of figure, row H). These measurements were taken from 1-μ-thick plastic sections stained with osmium tetroxide and toluidine blue. Within each square depicted on the figure, cells were counted going from medial to lateral. That is, the medial 100 μ is shown on the left side of each box, and each bar shows the next 100 μ more laterally. The most anterior cells in and around the suprachiasmatic nucleus appear in the left column of squares, and the most posterior in the right column of squares. If half or more of a particular 10,000 μ2 area contained SCN cells, the bar depicting that area was underlined. Some blank spaces appear on the graph (e.g., in row A). These loci near SCN contained primarily fibers from the optic chiasm or tractus infundibularis ventral and medial to SCN respectively. Note two features: 1. In general, the cell density is greater in SCN (bars underlined) than in the anterior hypothalamus adjacent to SCN. 2. The medial SCN has a higher cell density than the lateral SCN. This is particularly noticeable in the dorsal SCN (rows G, F, E).

neurons from astrocytes reliably; this problem is compounded in SCN because of high cell density, interpositioning of astroglia and neurons, and the fact that the neuron cell bodies are no bigger than those of astroglia. Therefore, taking these factors into consideration, the real number of neurons in our SCN material is less than the counted mean and can be estimated at around 8,000 per nucleus, or double for both nuclei. This number is consistent with previous measurements (Raisman, unpublished, cited in Guldner, '76; Riley and Moore, abstract, '77).

Cell size
Cross-sectional areas of neurons in different regions were measured with a Numonics Digital Planimeter from photographs magnified 4000 × taken from 1-μ sections from three brains embedded in Epon. One hundred five
neurons were measured from three different SCN regions from each of the three brains. The area was measured in neurons where the nucleus was visible. In neurons with a single nucleolus, the presence of the nucleolus in a single section as a criterion for measurement increases the probability that the measurement is made at the maximum diameter of the neuron. However, since SCN neurons generally have multiple nucleoli, with each nucleolus at the perimeter of the nucleus instead of the center, the presence of the nucleus provides the same advantage as nucleolus presence in ascertaining the middle of the cell. The mean area of cells in the dorsomedial SCN was 83.5 ± 4.1 (SD) μ², lateral SCN 108.4 ± 1.5 (SD) μ², and ventrolateral 102 ± 5.3 (SD) μ². Similarly, four paraffin-embedded rat brains were cut in 12-μ horizontal sections, Nissl-stained, and analyzed with a Zeiss Particle Size Analyzer. The mean diameter of 50 cells from each animal in the dorsomedial cell group was 7.83 ± 0.91 (SD) μ and from lateral SCN 9.58 ± 1.54 (SD) μ. Additionally one mouse and one hamster brain were paraffin-embedded and horizontally sectioned. In the mouse the medial SCN cells were 7.0 μ in diameter and lateral, 8.09 μ; similarly in the hamster, medial cells were smaller, 7.85 μ, than lateral cells, 8.69 μ. The above cell sizes are useful for comparative purposes, but because of section thickness, are an underestimate of true neuronal size (Paliovits and Fischer, '68). The rat cells within any one region of SCN are not homogenous in size. Ultrastructural analysis revealed large cells with a rich and complex cytoplasm interspersed with small cells with scant organelle-poor cytoplasm (Fig. 4).

In the Fischer rat, the majority of SCN neurons have multiple nucleoli. Of 250 cells examined in ammoniacal silver preparations of SCN, 193 (77%) had two or more nucleoli. In cells with multiple nucleoli, the nucleoli are situated on opposite sides of the nucleus near the nuclear membrane. In cells with a single nucleolus, it is generally situated in the middle of the nucleus. Additionally, a large number of SCN neurons, as well as astrocytes, have invaginated nuclear membranes (Fig. 5). These invaginations may be found as single indentations into the nucleus, or as a series of invaginations (Fig. 5). The infolding of the nuclear membrane increases the ratio of nuclear surface area to volume; with multiple infolding this ratio may be double that of a non-invaginated nucleus. Infolding also serves to decrease the distance between nuclear membrane and nucleolus.

Soma-soma appositions

In the dorsomedial SCN significantly more soma-soma appositions are found at the light and ultrastructural level than in the other areas of SCN. With light microscopy in the dorsomedial SCN with an average of 22.2 ± 3.5 (SD) cells found per 10,000 μ² in 1-μ plastic sections, 10.3 ± 5.2 (SD), per 10,000 μ², neurons had somata in apposition with other somata, while in the SCN immediately lateral to the dorsomedial area, where 11.8 ± 3.3 (SD) cells were found per 10,000 μ² area, only 0.8 ± 1.0 (SD) appositions were found per 10,000 μ². This quantitative analysis utilized one section at a time. Examination of serial 1-μ sections revealed additional cell appositions on cells which in a single section seemed isolated. This would suggest that in the dorsomedial SCN the percentage of cells having perikaryal contacts as calculated above would be an underestimate of the real number of cells having such appositions. In a 1-μ section it is unlikely that one cell would obscure another leading to a false impression of apposition; this problem would be more likely in thicker sections.

The mean length of the soma-soma apposition measured with a Numonics Digital Planimeter from a total of 181 appositions in the dorsomedial SCN subpopulation is 8.37 ± 2.29 (SD) μ. Since these figures are derived from light microscopy, it is likely that in some cases what is perceived as a soma-soma apposition has in reality a thin glial process between the two apparently opposing membranes. Appositions which are analyzed at the EM level show several variations (Fig. 6): Some cells are apposed over less than 1 μ to 11 μ; serial sections through cell appositions revealed appositions with up to 89 μ². Other cells are separated by axons or dendrites between somata. In other cases two neuron perikarya were segregated by one to six glial sheaths (Fig. 6E). The glial process between two adjacent neurons is generally thin with little cytoplasm in the process (Fig. 6C, 6D). The width of the process, measured from inner membrane leaflet to inner leaflet, varies from 30 up to 1,000 nanometers, generally showing a tendency toward the thinner shape. The width of the glial interface remains fairly constant between two neurons (Fig. 6C). When neurons are closely apposed, there is often a glial element at both sides of the soma-soma apposition. Apposed neurons that do not have a glial process between them may exhibit one of several intercellular membrane specializations or may simply maintain a 20-nanometer space between outer leaflets of the two membranes, as normally
Fig. 4. This electron micrograph shows several common ultrastructural features of SCN. One neuron (N₁) is significantly larger and contains a richer complement of organelles than the other neuron (N₂). Part of another large organelle-rich neuron (N₃) is seen in the upper right. An astrocyte of a size similar to neuronal size is immediately adjacent to the small neuron with which it maintains a large common border. The cytoplasm of SCN astrocytes is often unusually rich and contains a large number of Golgi apparatus, ribosomes, and rough surfaced endoplasmic reticulum. Glia fibrils running in parallel groups leave the edge of the cell body. The nuclear density of the astrocyte is significantly greater than that of the two neurons, but less than that of the oligodendrocyte in the lower left. 15 μ.
Fig. 5. A. Camera lucida drawings of invaginated nuclei from one-micron plastic sections in SCN. Invaginations such as these can increase the ratio of surface area to volume of nucleus by a factor of two. SCN neurons also have round or ellipsoid nuclei, but these are not represented here. Bar, 10 μ. B. Arrows indicate invaginations. While in two dimensions these oblong bodies look membrane bound, in three dimensions, out of the plane of section, they are fingers of cytoplasm with continuity to the non-nuclear cytoplasm. Here the three invaginations are parallel with one another. 8 μ. C. Two of the three invaginations in this neuron are parallel, while the third is at a right angle to the others. In both this cell and the previous one the nucleus is at one side of large perikaryon. 10 μ.
Fig. 6. A. A glial process separates two close neuronal somata. One perikaryon has a subsurface cistern adjacent to the glial process; the inner surface of the cistern has some closely associated ribosomes and is near rough endoplasmic reticulum. 1.2 \( \mu \). B. Two neurons are joined by an attachment specialization (triple arrowhead). This partly involves the base of a somatic spine. Also found between the perikarya is a segment of an axon and bouton, which makes synaptic contact with the neuron on the right. Attachment plates are seen holding the axon to both somata (double arrowhead and single unlabeled arrow). 2.6 \( \mu \). C. Two neurons have a closely associated glial cell on their left; the glia sends a thin process to divide the two neurons. 6.5 \( \mu \). D. Same as C, except that glial nuclear staining density is less here than in C. 5 \( \mu \). E. Two somata are divided by three astroglial sheaths. 0.4 \( \mu \). F. When two neural somata are directly opposed, the intercellular cleft may have an electron-dense material in it. The arrow indicates an area where the membranes have broken; interestingly, both membranes broke at the same place, indicating that some adhesive substance may be holding them to one another. 0.8 \( \mu \).
found between elements of the neuropil. In some cases the extracellular space between cells is of greater electron density than between other cells; this intercellular cleft may contain material that can act in some capacity to hold the two apposing membranes together. In brains that were treated harshly during fixation, the membranes between two cells broke in several places but, interestingly, the break occurred in the same place in both membranes, which remained together in spite of obvious distortion in nearby loci (Fig. 6F). This suggests some intercellular adhesive. Some somata have punctate densities between them, probably serving as attachment plaques. These attachment plates occur individually or in groups (Fig. 7C). One unusual set of attachment plates occurs in short series of four to six densities with regular intervals between them (Fig. 7A). Because of their similarity to zippers, they will be referred to as zipper junctions. Each punctate density is separated from its neighbor by approximately 0.1 micron. Zipper junctions are found between somata and sometimes between somata and axon terminals. Where zippers are found between cell bodies, a high density of mitochondria is sometimes seen. Smooth endoplasmic reticulum can also be found, which appears to be in possible contact with the dense structure (Fig. 7B). Occasional synaptic clusters may be connected by a slightly similar series of more randomly spaced punctate junctions (Fig. 7D).

The EM analysis of soma-soma appositions revealed that close appositions occur not only between neurons but also between neurons and glia (Fig. 4) and between glia. Appositions exist not only between two cells but also between groups of cells organized in chains of cells; these chains are most often oriented in a rostrocaudal direction (Fig. 8).

Axons

Stains (e.g., Luxol blue) for myelinated fibers show only a few in the SCN. Tissue prepared with Bodian's ('36) protargol method shows relatively few stained fibers within the SCN compared with the region immediately lateral to SCN where, in a horizontal section, many fibers are seen running in a rostrocaudal direction (Fig. 1B). Within the SCN fibers tend to be small and unmyelinated, ranging in size down to 0.15 μ. Occasionally bundles of several hundred axons are seen composed primarily of unmyelinated axons with a few myelinated axons (Fig. 11).

Golgi impregnations of neurons within the SCN show a large number of short axons. Axons originate from either a perikaryon (Fig. 12A) or from a proximal dendrite (Fig. 9A, B), although a few cases were seen where the axon arose from a large secondary dendrite (Fig. 12B). From a total of 88 cells in eight brains where frequent axons could be clearly seen, about half of the cells (51%) had axons that originated from the dendrite. The remaining 49% had soma derived axons. The mean dendritic length from the soma at which axons derived in the first group was 10.9 ± 7.5 (SD) μ. This was measured from the axon hillock to the junction of dendrite with perikaryon. Boutons from local SCN axons were seen terminating as close at 6 μ from the neuronal perikaryon of origin. Axons possess both boutons en passant and boutons terminaux. Single boutons are interspersed over a wide range. Small groups of three to six boutons derived from the same axon were seen contacting a single postsynaptic element, either a dendrite or soma.

Axons originating from SCN cells were examined that maintained up to 17 branching points. This number is probably an underestimated value; additional branches may have resisted silver chromat impregnation.

Dendrites of short axon cells covered a greater distance than did the axons of the same cells. This was determined by measuring the distance from axon hillock to the bouton farthest away from axonal origin. Dendritic spread was measured from the terminal ends of the two dendrites farthest from each other.

Axons were seen with both Golgi impregnations and filled with HRP that crossed the midline into the contralateral SCN either under the third ventricle or perpendicularly through the tractus infundibularis (Fig. 13B). These axons were very fine and stood out best with darkfield illumination. In cases where a small amount of HRP was iontophoresed into the lateral SCN (Fig. 9E), numerous cells in the contralateral SCN were backfilled with a granular HRP deposit suggestive of axonal uptake and retrograde transport (Fig. 9D). In an equal distance but lateral direction from the injection area 90% fewer labeled cells were found. Therefore the possibility seems unlikely that the cells in the SCN contralateral to the site of microiontophoresis were a result of simple diffusion from the injection locus.

Axons of undetermined origin are seen entering SCN from all possible directions: ventrally from the optic chiasm and supra- and post-optic commissures; posteriorly from the area of the mediodasal hypothalamus; and laterally, anteriorly, and dorsally from the surrounding anterior hypothalamus. Similarly, SCN axons are...
Fig. 7. A. Two neurons with apposed somata have an unusual series of punctate junctions between them. Two sets of these zipper junctions occur between perikarya; two additional junctions of somewhat similar morphology occur between axonal boutons and the soma of one of the neurons. 13 μ. B. Higher magnification of zipper junction seen in A. In close proximity to the junction are seen a number of mitochondria, and at several points, smooth endoplasmic reticulum appears to contact the intercellular junction. The short punctate densities appear in both neuronal somata and in the intercellular cleft. The mean distance between each small density remains fairly constant at ~0.1 μ. 1.8 μ. C. In addition to the above-described situation, single spot-like attachment junctions occur at irregular intervals between two apposed neurons. 2.1 μ. D. A cluster of axon terminals around a central figure (star) have short synaptic densities between pre- and postsynaptic elements. 1.0 μ.
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Fig. 8. Chains of cells, as seen here, are common in the dorsolateral SCN. Anterior is up in this horizontal section. These chains of cells involve predominantly neurons, although astroglia are also included. The length of the chain is most often directed in an antero-posterior direction. These groups of neurons are not restricted to a one-cell thickness, but may involve additional cells above and below the plane of focus. Large regions of somato-somatic apposition occur between cells involved in the chains. Reverse image of osmium-toluidine blue plastic section. 32 μ.

found leaving the nucleus in dorsal or posterior directions and, less often, laterally and anteriorly. Further analysis of SCN efferents is outside the scope of the present paper.

Based on silver chromate impregnations of axons within SCN, a number of distinct populations of axon arboris are found; these axons were of undetermined origins. The largest diameter axons may pass through SCN without terminating within it. These axons occur in loosely packed bundles (Fig. 10A). Six more types of axons are seen in Figure 10. All of these maintain boutons in the SCN, both terminal and en passant. The thinnest axons found in Golgi material are only visible as a series of thin, unconnected dots arranged in a linear or branching pattern. These have sparsely arborizing terminal fields and very small boutons (Fig. 10B). Other axons may be relatively thick with many symmetrical and asymmetrical varicosities, some of which represent boutons en passant. While some axons have few impregnated collaterals, others (Fig. 10B) possess an extensive number of branches and terminal boutons. Axons and boutons in the SCN will be described more fully in a subsequent paper. Since axons of 0.15–0.20 μ diameter are seen in electron micrographs, a size near the limit of resolution of the light microscope, there may exist one or more types of axons which either 1) are not impregnated by the Golgi reaction or 2) are too thin to see in thick sections with conventional light microscopy.

**Axon fascicles**

Groups of axons or fascicles are a common feature of the SCN neuropil. These are seen with 1-μ toluidine blue sections, Golgi impregnations, the Sevier-Munger ('65) stain (Fig. 11A), and with electron microscopy (Fig. 11B). These fascicles are composed primarily of small unmyelinated axons with a few thinly myelinated axons interspersed. The ratio of unmyelinated to myelinated axons in the fascicles is about 12:1, based on counting the axons in randomly generated electron micrographs. The number of axons in the fascicles varies between 50 and several hundred. Diameters of the axons range from 0.15 μ for the smaller unmyelinated axons to 2.1 μ for the larger myelinated axons. In Golgi material some of the axon bundles are seen arising from the ventral supraoptic commissure that runs ventral to the SCN. Groups of axons leave the commissure and proceed in a dorsal direction. Axons tightly grouped together may spread apart like a flower spray within SCN (Fig. 11A). Other bundles of axons traverse the SCN, originating ventrally and emerging dorsally, presumably to terminate in other loci.

**Local circuit axons**

Short axons or axon collaterals arising and terminating in the SCN are a common feature of the nucleus. Locally terminating axons are found arising from the majority of neurons in which at least 50 μ of impregnated axon could be followed. These arise from perikarya (Fig. 12A), the junction between dendrites and somata (Fig. 12C), and from dendrites. The dendritically derived axons come either from a bend in a proximal dendrite (Fig. 9A), as described in the substantia gelatinosa (Gobel, '75), or from the shaft of a proximal dendrite (Fig. 9B). Four examples were seen where the
axon emanated from a secondary dendrite, one at a distance of 36 µ from the junction of the cell body and the base of the dendrite (Fig. 12B). Some local circuit axons terminate diffusely throughout the SCN while others maintain a more restricted field of boutons near the cell of origin. Short axons have both terminal and en passant boutons that abut on dendrites, dendritic appendages, and somata of SCN neurons. Although some short axons that terminate in SCN have short collaterals that end in the anterior hypothalamus outside the SCN, these are found only rarely. The majority of local circuit axons do terminate within the boundaries of SCN. From three to over 100 boutons can be found within the SCN originating from a single axon. It is probable that in cases where only a few boutons were seen, the axon was incompletely impregnated or ran out of the section. The possibility that locally terminating axons are collaterals of long projection axons is examined in the Discussion.

A total of six cases in several hundred brains were observed in Golgi material where a very short axon appeared to contact dendrites of the cell of origin; these have been seen only in young rats less than one month of age. One example of a possible autapse in an eight-day-old rat is seen in Figure 9C. It is conceivable that the contact is made by a growing axon as SCN is still undergoing synapse formation at this time (Lenn et al., '77); this axon may withdraw from its dendrite at a later stage of development.

Dendrites
Neurons within the hypothalamus are relatively difficult to segregate into cell types on the basis of gross structure. In other neural loci, such as the olfactory bulb, or the cerebral and cerebellar cortex, not only do the cells have distinct laminae, but the dendritic ramifications are in most cases easily recognizable. Neurons in the hypothalamus, however, tend to
Fig. 10. Segments of Golgi impregnated axons. A through G were all drawn with the same camera lucida magnification from sagittal sections of Golgi impregnated adult rats. Axon diameter in A through G is relative but, because of the inherent difficulty in drawing thin fibers with camera lucida, it may not be exact. Bar, 25 μ.

A. Bundles of relatively large axons that may either break up into local boutons or enter and leave the SCN without any apparent SCN collaterals. These axons take a slightly wavy form, and are the thickest seen in the nucleus with the Golgi method. The thinnest axons found, these fibers are seen as a series of almost connected dots and dashes that make occasional short lateral offshoots. C. The thickest axons with local boutons. The barrel of the axon is covered with varicosities, some symmetrical, but more often asymmetrically situated on the axon, and probably representing boutons en passant. D. These fibers are seen continuing outside the SCN. Within the nucleus, they travel in parallel groups with occasional side branches projecting into the SCN. Occasional varicosities are seen along the stem of the axon. E. These axons have the most side branches, collaterals, and the largest terminal boutons. Unlike A and D, these axons seldom maintain a straight course but curve and curl through the neuropil. Single long strands up to 12 μ long coming from a thicker part of the axon terminate in a single swelling. F. This axon type has some of the characteristics of C but is generally thinner and has smaller varicosities. Additionally, thin branches with small varicosities may emanate from the main twisting axon and proceed for 10–20 μ. G. Like F, the main part of the axon twists frequently; thick side branches proceed laterally, some thinning out and others ending in a small terminal bouton. H. Montage of coronal section of adult Golgi-impregnated brain. In blocks such as this where axons are well impregnated, but cells are not, a plethora of axons in the anterior hypothalamus surrounding the SCN is found, while within the SCN axons are less frequently impregnated. Bar, 80 μ.
possess relatively simple dendritic structures and, compared to cortical structures, no easily recognizable strata can be found. Furthermore, when dealing with a relatively simple population of neurons, one runs the risk of confusing variants on a theme with neurons that are truly morphologically different. These complications notwithstanding, several cell types can be seen even in a low magnification of Golgi material (Fig. 13). These types are seen in several different methods of Golgi impregnations and appear to be distinct from one another. That is not to say, however, that the cells in the different categories are necessarily distinct functionally.

Based on the dendritic trees of material examined, neurons of SCN can be divided into several classes. All are relatively simple in comparison to pyramidal cells of the cortex or Purkinje cells of the cerebellum. For the most part dendritic fields are smaller in SCN than in either the ventromedial nucleus or the lateral hypothalamus. The least differentiated cell in the SCN, that can be designated simple bipolar, has two primary dendrites at opposite ends of the perikaryon, giving the neuron a fusiform or bipolar shape. The dendrites may not branch at all (Fig. 14A) or they may branch once or twice (Fig. 14B), usually at the distal part of the dendrite. Spines are either absent or rare. Dendrites tend to stay in a fairly linear orientation, seldom doubling back or diverging much from the original course of the proximal dendrite. If the soma is found at the periphery of the SCN, dendrites may curve parallel to the general curvature of the nuclear boundaries. These simple neurons are found commonly in two areas of the SCN. In coronal sections, they lie dorsal to the optic chiasm or supraoptic commissure, with the dendritic elongation perpendicular to the midline. The medially directed dendrite proceeds toward the midline where it may turn dorsally. A second place within the SCN where the simple neuron is commonly found is in horizontal sections, close to the midline, with dendrites running parallel to the tractus infundibularis of Krieg ('32). With rapid Golgi impregnations axons can be seen in horizontal sections that course from the anterior lateral part of SCN and enter SCN anteromedially with short side branches.
Fig. 12. Three neurons from ventral horizontal section of nucleus. Axons coming from the neurons show the three types of axonal origin. From A, the axon originates from the cell body, near a dendrite that was not followed out of the Golgi section (indicated by a short line perpendicular to the orientation of proximal stump of dendrite). Asymmetrical boutons en passant are found on the axon. Neuron B is one of a handful of cells found where the axon comes from a dendrite distal to a bifurcation in the dendrite. Boutons en passant are not found on this axon; several short side-branches are seen along the irregular course of the main axon. The axon of neuron C originates at the junction of the cell body and a dendrite. Beside the arrow indicating the axon origin, part of a small dendrite can be seen. Notice the dendrite indicated by the dark triangle; it tapers off into a very fine thread-like distal ending. If not found connected to the dendrite, this very thin distal part could be mistaken for a short segment of axon. However, the distal dendrite of cell B, indicated by the dark circle, tapers gradually but does not end in such a fine structure. The axon from cell A does make contact with the dendrite of cell C. At one point the axon of cell C crosses its own dendrite; there is no contact here. 20 day. Bar, 50 μ.
Fig. 13. Horizontal section of left and right SCN. A number of impregnated neurons can be seen, and a few astrocytes are visible. A. Part of the optic chiasm is on the left. This section is ventral to the area where the third ventricle bisects the paired SCN. The star indicates two cells of which higher magnification drawings are seen in Figure 16B. In this block more neurons are impregnated in the medial SCN than in the lateral part of the nucleus. A number of small round structures are impregnated; these are nuclei of neurons and glia (see EM in Fig. 18F, G, H). 8 day. B. Very thin HRP-filled axons cross the midline (shown with triangles) from the left SCN into the right SCN. HRP had been iontophoresed just lateral to arrow; the injection site is shown in Figure 9E. Axon thickness is exaggerated to facilitate visibility. Camera lucida drawing from horizontal section. Bar, 85 μ.
Fig. 14. A. Horizontal section adjacent to the midline. Tractus infundibularis is marked by a row of black triangles. The dark neuron is representative of the simplest type seen in SCN, characterized by two simple dendrites that project from opposite ends of a fusiform cell body. The dendrites maintain a fairly constant orientation and do not have a large number of appendages. A number of neurons in this region of SCN possess dendrites that are parallel to the tractus infundibularis. The dotted neuron is a slightly more elaborate form of the black one; it has a short dendritic branch near the cell body and a number of small dendritic appendages. 20 day. Bar, 50 μ. B. A common variant of the simple bipolar cell; this one has two primary dendrites, one of which branches. Both branches proceed in the same general direction. The neuron in Figure 12B is similar, but both of its primary dendrites branch once. 8 day. Bar, 100 μ. C. Neurons with only one dendrite (monopolar) leaving the cell body represent less than 3% of the total number of neurons in SCN. These two from a coronal section in the posterior part of SCN are typical of such cells, having a thick primary dendrite that bifurcates at least once near the soma. 8 day. Bar, 50 μ.
and boutons en passant in SCN; these axons leave SCN posteromedially. The terminal area within SCN of these axons of undetermined origin is in the same area as the simple bipolar cells whose dendrites run parallel to the main trunk of the axons here. As somata and dendrites were not impregnated in brains where these axons were found, it is not presently possible to determine if the axons terminate selectively on the simple cells here.

A small number of cells have a single primary dendrite (Fig. 14C); the trunk of this dendrite is generally somewhat thicker than each of the dendrites found in multipolar cells. The main trunk breaks up into as many as four smaller distal dendrites with the first bifurcation within 5–20 μm from the perikaryon. A close relative of the monopolar cell is one that has a large dendritic trunk that breaks up into smaller distal dendrites on the side of the soma, and additionally has a single thinner dendrite deriving from the opposite side of the cell body (Fig. 12C).

A third type of a cell is the curly bipolar (Figs. 12B, 14B, 15A). Spines are found on the dendrites and sometimes on the soma. Each of the two primary dendrites may branch once or twice. Dendrites of the curly bipolar are more likely to alter direction from their initial trajectory than dendrites of the simple bipolar.

A fourth cell type in SCN, the radial multipolar, has from three to five primary dendrites that extend from the perikaryon like the spokes of a bicycle wheel (Fig. 15B). These cells are most commonly found in the dorsolateral and anterior SCN, often in the transition zone at the edge of SCN.

A fifth type of neuron in SCN is the spiny neuron (Fig. 16A, B). The cell body is often quite round. The dendrites have many appendages of varying shapes and sizes, ranging from a modest thorn to long strands, lollipops, and mushroom shapes, dumbbells, and two stalks emanating from the same dendritic varicosity. These cells often have a large number of protuberances arising from the perikaryon. Lengths of appendages range from one micron to unusually long spine-like strings extending 10 μm from their point of origin. Appendages are sometimes seen bending over in such a fashion that the distal end of the appendage may abut the parent dendrite (Güldner and Wolff, '78) presented ultrastructural evidence that spines in the SCN may be presynaptic to the parent dendrite; spines whose distal end appears to contact the parent dendrite are not uncommon in Golgi impregnations of the SCN in both young and mature rats. Spines such as these are also seen in the magnocellular paraventricular nucleus in young rats.

Dendritic appendages (Figs. 17, 18) receive a significant number of synapses in SCN. As mentioned above, some may be presynaptic. While the appendages of the spiny neuron have already been discussed, with the exception of simple bipolar cells, other neuronal types have several kinds of appendages. These range from thin stalks with or without an end swelling (Fig. 17A–D, 18A, D) to more complex types with several round swellings either centrally placed (Fig. 17C) or laterally positioned on the spine (Fig. 17B). Frequency of spine occurrence varies from several per linear micron of dendrite (Fig. 18D) to one per 10 or 12 micra. As can be seen in the electron micrograph of a dendrite with many spines, the spines greatly increase the dendritic surface area. Some dendrites do not have obvious spine-like appendages but may have very irregular contours (Fig. 18C) with large round or sharp varicosities along the dendrite. Golgi impregnations were significantly more successful than local extracellular HRP iontophoreses (Fig. 17E) in labeling dendritic appendages. While appendages described are found in both young rats and in adults, the distribution and size may vary with age.

To clarify an unusual type of impregnation, it should be pointed out that the dark circular objects seen in Figures 9A, C, 13, 17B–D are not cross sections of large impregnated dendrites or impregnated cell bodies. With ultrastructural examination they were seen to be impregnated nuclei of neurons and astroglia. Impregnated cell nuclei were seen only with one Golgi method which utilizes dimethylsulfoxide and chloral hydrate.

In Golgi impregnations one sees a number of cases where one dendrite comes into apparent contact with another from SCN. With ultrastructural analysis, dendrodendritic synapses can be found, in agreement with earlier observations of Güldner ('76; Güldner and Wolff, '74).

In general, dendrites of SCN cells tend to stay within the nucleus; this is especially true of the medial and ventral SCN. Dorsally and laterally, dendrites can be found that leave SCN cells and proceed into the adjacent anterior hypothalamus. Similarly, dendrites of cells situated dorsally and laterally to the suprachiasmatic nucleus occasionally extend into the SCN. Infrequently dendrites of SCN cells are found in the optic chiasm, as reported in mice.
Fig. 15. A. Dendrites of these two cells, especially the grainy one, are more likely to alter their direction after the initial segment. More frequent and larger spines are seen on the dendrite of the cell on the top. The dendrites change direction soon after leaving the perikaryon. The locally terminating axon arises from the initial bend in the dendrite (dotted cell) or immediately distal to the first bend in the dendrite (black cell). The axon of the black dendrite has some terminals that end within a few micra of the cell body and dendrites. The cell bodies seen in the ultrastructural presentation of Figure 5B and 5C may be from neurons similar to the dotted one here. In these cells the perikaryon is seemingly extended by the large trunk of the initial dendritic segment, and the nucleus may appear in the cytoplasm opposite the emergence of the dendrite. 20 day. Bar, 50 µ. B. This neuron is typical of some found at the periphery of SCN. It maintains the greatest number of primary dendrites seen in SCN (three to five). These dendrites leave the cell body in a manner similar to the spokes of a wheel. Part of the axon is indicated by the arrow. Adult. Bar, 50 µ.
Fig. 16. A. This neuron and the black one in 16B have the most complex and longest dendritic appendages, reaching more than 10 μm in length. Some are short and stubby, while others may be thin and long. The cell body is generally roundish, and two primary dendrites project from opposite poles of the cell. Appendages are found on the cell body and look similar to those seen on the proximal and distal dendrites. 20-day Bar, 50 μm. B. The dotted cell here is one of a dozen found. The processes are fairly short even for the SCN, where dendrites are generally short. A number of extensions from both the cell body and processes give this cell a total length not too dissimilar from those with two long dendrites with few appendages. 8-day Bar, 30 μm.
Fig. 17. A. Horizontal section showing a Golgi-impregnated neuron with various spines and appendages. The arrow indicates a dendrite with fairly large appendages of different shapes and sizes. Tractus infundibularis is densely impregnated at the top of the figure. A number of axons can be seen, especially in the lower right. A translucent blood vessel runs under the soma of the neuron. Adult. 170 μ. B. Neuron has a number of complex appendages; one, indicated by arrow, has a small spherical structure on the side of a thin strand. 20 day. 80 μ. C. The dendrite running horizontally across the top has primarily spines with a short thin stem ending in a round top. Another dendrite has a long strand-like spine with several swellings along it. 20 day. 40 μ. D. A number of spines and branches are seen on these dendrites. 8 day. 45 μ. E. HRP extracellular iontophoresis. Labeled dendrite shows no spines; seldom were spines seen with HRP. A number of HRP-filled axons are seen in the lower left. Adult. 60 μ.
Figure 18
of 64,564 hypothalamic ventromedial nucleus, drawn fundibularis, and post-(supra)optic commissures. The largest process of SCN astroglia, heavy retinal input to SCN is situated. If the well over a hundred terminal branchlets (Fig. 19A) branch several times, giving a single astrocyte found arising from the astroglia soma. These were not measured. These numbers, though were not measured. These numbers, though underestimates of real total dendritic length, serve to demonstrate the small size of dendritic arbors in SCN compared with other hypothalamic loci.

**Astroglia**

The cell bodies of astroglia in the SCN are about the same size as neuronal perikarya as seen with Nissl stain, EM, or Golgi impregnations. The largest process of SCN astroglia, as shown by Cajal's ('13) gold sublimate stain in 35-µm sections, invariably projects toward a nearby blood vessel with which it makes contact (Fig. 19A). Five to 15 other processes are found arising from the astroglia soma. These branch several times, giving a single astrocyte well over a hundred terminal branchlets (Fig. 20A). Processes of astroglia stained with Cajal's gold sublimate method (Fig. 19A) did not show the finer detail seen with Golgi impregnations of astrocytes in thick sections (Fig. 20A), nor were as many fine processes seen with the Cajal stain. This is partly due to the thinner section used for the gold sublimate stain. If, as suggested by Vaughn and Pease ('67) and Mori and Leblond ('69), Cajal's stain works by staining glia fibrils, the reduced detail of the method may be due to the paucity of fibrils, as seen with electron microscopy (Fig. 20B), in smaller extensions of main processes.

Astroglia within the SCN often have branches packed with bundles of filaments running parallel to the main course of the glial arm. While the bulk of the main branch of the glial arm may be filled with filaments, lateral bulges along the glial process contain a significant number of organelles including mitochondria, lysosomes, smooth- and rough-surfaced endoplasmic reticulum, polyribosomes, and occasional multivesicular bodies (Fig. 20B). These lateral bulges are also the site where smaller thin processes spread through the adjacent neuropil segregating neuronal elements (Fig. 20B). Spine-like protrusions on large glial processes are seen with both Golgi impregnations and electron microscopy.

The typical astroglial cell in SCN does not fit readily into either the protoplasmic or fibrous category. Like protoplasmic types, astrocytes in SCN are found within gray matter and, similar to fibrous types, astroglia often have bundles of filaments coursing parallel to the main processes. The divisions of astrocytes into fibrous or protoplasmic categories may be somewhat arbitrary, as suggested by Jones and Cowan ('77); both categories may be variants of the same type. Differences may be the result of local microenvironment.

In a number of EM specimens gap junctions were found between astrocytes. Tissue that had
Fig. 19. A. Camera lucida drawing of 35-micra horizontal section stained with Cajal’s gold sublimate method. Five astrocytes are seen in this section, and should be compared to the thicker section of a Golgi-impregnated astroglia in Figure 20A. Large processes from three of the astrocytes are seen where they contact nearby blood vessels (stars). Thinner processes project through the unstained neuropil. Bar, 50 μ. B. Two glial cells with cell bodies situated in the optic chiasm and tract send some processes dorsally into the SCN. Boundaries of SCN are indicated with triangles. Glial processes such as these extend into the ventrolateral SCN, the same area of SCN that receives retinal input. Bar, 100 μ.
Fig. 20. A. Golgi-impregnated astrocyte from 100-microi section. More processes can be seen here than with the Cajal stain (Fig. 19A) because of the thicker section here and because of histological staining differences. Thin processes end with a small flattened or round bulb, or without any specialization. Adult, Bar, 20 μ. B. EM of a large process leaving the cell body of an astrocyte; this process contains a bundle of fibrils coursing parallel to the main trunk of the glial process. Varicosities (large arrowheads) bulge out laterally from the main trunk and contain a number of organelles including ribosomes, mitochondria, rough-surfaced endoplasmic reticulum, electron-opaque vesicles, and other membrane-bound bodies. From these lateral bulges several fine processes extend into the neuropil (small arrows). The organelles found in the lateral bulges of the glial process suggest some protein synthesis may occur in the glial process. 2.6 μ.
been block-stained with uranyl acetate or tannic acid best illustrated the typical junction as described by Brightman and Reese (69). Gaps consisted of two membranes closely apposed with an intercellular space of 2.0–2.3 nanometers. The junction consists of four densely stained membrane leaflets interposed with three layers of lightly stained spaces, resulting in a seven-layered structure typical of gap junctions. The membranes assume a fairly rigid posture and maintain a constant interval between them.

Communicating junctions were found between astrocyte processes that surrounded blood vessels (Fig. 21G, H), where the junction is up to three micra long. Other cases were found where gap junctions occur between glial elements in the neuropil. In single sections, gaps in neuropil varied from 200 to 2,400 nanometers in length. The largest gap junction found was between two astrocytic processes in the ventral SCN. The gap appeared in 53 serial thin sections (Fig. 21A–E), with a maximum length of 2,400 nm on a single section. As the junction apparently continued farther than the serial sections showed, assuming a section thickness of about 0.08 μ, the cross-sectional area of this gap was calculated to be in excess of $5 \times 10^6$ square nanometers, or 5 μ².

The use of lanthanum nitrate as suggested by Revel and Karnovsky (67) showed the honeycomb appearance described in gap junctions from other loci when the junction is cut en face (Fig. 21F). The center-to-center distance of each pore measured from lanthanum-treated brains is approximately 9.0 nm, similar to gap junctions in other loci.

**DISCUSSION**

Besides its position in the hypothalamus dorsal to the optic chiasm and supra- and postoptic commissures, and lateral to the ventral aspect of the third ventricle, the suprachiasmatic nuclei are distinguishable from other hypothalamic nuclei by several factors: One is the tight packing density of its small neurons, which are among the smallest in the brain. Another is the frequent long perikaryal appositions. With certain protargol stains, the SCN stands out by its apparent paucity of axonal staining, compared with other hypothalamic areas where axons are readily visible. This is also seen in those Golgi impregnations in which predominantly axons are stained.

**SCN subdivisions**

Despite the functional importance of the hypothalamus, our understanding of its intrinsic organization has lagged far behind our knowledge of other neural loci. One problem that hampers anatomists is the apparent lack of a readily understandable organization in the hypothalamus. One of the first steps to piece together the hypothalamic puzzle is to separate each hypothalamic nucleus into local subdivisions and into cell types. From this information we can begin to try to analyze the circuitry and interaction within and between hypothalamic nuclei.

Palkovits and Fischer (68), Szentagothai et al. (72), and Krieg (32) suggested that the SCN was a homogenous population of small cells. With closer examination, the present study finds striking subpopulations of cells within the SCN (Fig. 3). Although other minor subpopulations exist, two predominant ones are designated the dorsomedial group of cells and the ventrolateral group of cells. Compared with the ventrolateral cell group, the dorsomedial group is characterized by its smaller, more tightly packed cells and the frequent intermingling of neurons and astroglia. Previous studies used a coronal plane with thicker sections than the present study which utilized horizontal plastic and paraffin-embedded sections. Neurons of the ventrolateral SCN show a significantly greater number of invaginated nuclei than cells of the dorsomedial part; additionally, a greater frequency of cells without invaginated nuclei contained marginated

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**Fig. 21.** A–D. Serial sections through a gap junction between astrocyte processes in the neuropil. Fifty-three serial sections were cut from a Golgi-impregnated block stained en bloc with uranyl acetate. No Golgi-impregnated structure is seen in these particular views. The gap junction was followed continuously from the first to the last of the 53 serial sections. The serial section number of four of the sections is found on the right of the dark square in the lower left of each photograph. In A and B a single short gap connects two glial processes. Several axons are seen in the near vicinity, filled with vesicles. An attachment plate is seen on the left of each gap junction. In C, the length of the gap has increased considerably, and an additional gap junction is found underneath and almost touching the first. In D the second gap junction has increased in size (on the right) and the original one is cut at a more oblique angle. A, B, 1.1 μ; C, 1.8 μ; D, 0.9 μ. E. Larger magnification of D; the small gap between adjacent membranes is seen at the point of the small arrow. F. Extracellular lanthanum nitrate shows the hexagonal array of a gap junction. The center-to-center spacing of each pore in this figure is ~9 nanometers. 0.1 μ. G. A gap junction between two glial processes just outside the basement membrane of a blood vessel. 0.3 μ. H. Higher magnification of G. Again, as in E, the typical structure of the gap can be seen, with the cleft about 2 nanometers wide.
Figure 21
nucleoli (non-centralized) in the dorsomedial part (Armstrong, '77). It is already known that the afferent projections to SCN tend to segregate into the SCN subnuclei. Interestingly, the majority of substantial input to SCN terminates on the larger and less densely packed cells of the ventrolateral SCN. This is true of retinal input (Hendrickson et al., '72; Moore and Lenn, '72), raphe input (Fuxe, '65; Aghajanian et al., '69), and input from the ventral lateral geniculate (Swanson et al., '74; Ribak and Peters, '75). Division of SCN into subpopulations of neurons is further supported by studies of hypothalamic cytogenesis with tritiated thymidine labeling (Altman and Bayer, '78); cells of the ventrolateral part of SCN undergo their final mitosis before neurons of the dorsomedial SCN, and a similar gradient exists in the rostrocaudal axis with rostral cells of the dorsomedial SCN, and a similar gradient exists in the rostrocaudal axis with rostral cells developing before caudal ones.

A computer-assisted procedure has been used to locate the critical neural locus in the SCN area in which radiofrequency lesions eliminate circadian rhythms of feeding and drinking (van den Pol and Powley, '79). Additional analysis of those data revealed that destruction of the medial SCN, especially the dorsal and posterior part and immediately dorsal to SCN (Fig. 7B in van den Pol and Powley, '79), caused the greatest reduction in the autocorrelation coefficient, a measure of circadian rhythmicity (van den Pol, unpublished data). This would indicate that either cells in, or axons passing through or near, the dorsomedial SCN are a critical factor in circadian rhythms. However, another possibility that cannot be excluded is that destruction of the medial area was linked in a non-random lesion localization that might artificially increase the apparent importance of medially situated loci within the general area involved in circadian rhythms. The ADH-neurophysin-positive cells tend to congregate dorsomedially and their axons leave SCN in several directions (Zimmerman, '76; Sofroniew and Weindl, '78; Vandesande et al., '75), raising the possibility that ADH-containing axons may play a significant role in communication between SCN and other neural loci. A study by Stephan and Zucker ('74) found that Brattleboro rats, which have hereditary diabetes insipidus and lack ADH, show an attenuated but distinct nocturnal pattern of drinking that is restored to normal with ADH injections spaced 12 hours apart. Thus under 12:12 light:dark conditions, a circadian pattern of ADH release by SCN is not necessary for manifestation of diurnal drinking rhythms. If further studies find that Brattleboro rats with constant levels of ADH replacement would show a free-running drinking rhythm under constant dark conditions, this would indicate ADH is not a critical factor in SCN's involvement in circadian rhythms.

While the majority of afferent input to SCN so far demonstrated terminates in the ventrolateral region, the dorsomedial part does receive a substantial innervation from neurons of the ventrolateral SCN and vice versa.

The neurons of SCN as determined by examination of dendritic arborizations obtained with Golgi impregnations can be divided into several types. One is the simple bipolar, characterized by a fusiform shape with two fairly straight dendrites extending in opposite directions. A second neuron is the curly bipolar; dendrites of these cells show a greater tendency to bifurcate and to curve away from their initial trajectory. Monopolar cells with a single large branching dendrite and spiny neurons with many large dendritic appendages are also found. A final type, the radial multipolar, has three to five primary dendrites radiating out from the cell body; these cells appear somewhat similar to neurons of the ventromedial nucleus (e.g., Fig. 8A, C, D in Millhouse, '73). While the neurons of SCN can be divided into these groups, a number of cells can be found within the nucleus that are of intermediate classification. All cell types have axons both on perikarya and emanating from proximal dendrites.

In general, the two to five dendrites of SCN cells tend to be fairly short, although some of the short axons are shorter than the dendrites of the same cell.

Dendrites of medial and ventral SCN cells tend to remain within the nucleus. Some lateral and dorsal cells send dendrites laterally into the adjoining anterior hypothalamus or dorsally into the periventricular region. Likewise, long dendrites from neurons of the anterior hypothalamus sometimes are found extending into the lateral and dorsal borders of the SCN, occasionally making contact with short axons of SCN cells. Dendrites from SCN cells may also be found projecting into the optic chiasm and tractus infundibularis. These data suggest that SCN dendrites may receive synaptic contact outside the nucleus itself; along these lines, then, SCN neurons may be influenced by axons and neurotransmitters not found within the SCN boundaries. Similarly, unless followed to the point of origin, it cannot be assumed that a dendrite within SCN, especially at the borders, arises from a SCN peri-
karyon. Experimental lesions restricted to SCN would thus destroy not only SCN cells, but would also injure dendrites of adjacent anterior hypothalamic neurons.

In some neurons in SCN dendritic appendages are not found, while in other cells, as seen with EM and Golgi impregnations, dendritic appendages are seen in a variety of shapes and sizes. These range from simple thorn-like protrusions to complex double spines with the appearance of barbells or Mickey Mouse ears. In both young and adult animals, some neurons possess dendritic appendages that are unusually long, up to 12 μ. The role of such long spines is not clear; they would have a high longitudinal resistance and would attenuate current movement caused by postsynaptic depolarization. This would serve to isolate the shaft of the dendrite from synaptic activity at the distal end of the spine and additionally would isolate the dendritic appendage from potential alterations in the dendritic shaft. With ultrastructural analysis, large areas of dendritic shaft are found that have a low frequency of synaptic specialization. As ample surface already exists, this would suggest that spines on these neurons are not needed simply to increase the amount of membrane available for synaptic contact. Autaptic spines are discussed later.

The present study used male rats for all histology except for Golgi impregnations, in which males and females were used in equal proportions. Brown-Grant and Raisman ('77; Raisman and Brown-Grant, '77) have suggested that the suprachiasmatic nuclei play a critical role in the estrous rhythm of female rats. Along these lines several papers have suggested morphological differences between the SCN of males and females. Gündner ('76) reported a higher frequency of multivesiculated bodies in female rats than in males. Greenough et al. ('77, abstract) reported differences in dendritic orientation in male and female hamsters. Clattenburg et al. ('72) suggested altered morphologies of SCN cells after coitus in rabbits. In the present study male and female SCN material was not compared.

Communication between SCN cells

Several morphological specializations are found that operate to hold membranes of apposed SCN neurons together. Single attachment plates are found between various elements of the SCN neuropil. These puncta adhaerentia are found in many other neural loci, and their primary function appears to be adhesive, holding together apposed membranes of different cells (Peters et al., '76). Serial attachment plates (zipper junctions) occur between adjacent SCN somata and may provide a more stable attachment than would a single punctum adhaerens. In certain cases where the tissue is treated roughly and signs of membrane breakage can be found, when the membranes between two apposed neurons are broken, the membranes maintain their typical 20 nm separation and tend to remain together even though several breaks in the membrane are evident. Although an occasional suggestion of a gap junction between two apposed neurons is seen, no systematic and convincing evidence for this has been found despite a continuing search.

A large number of neurons in the SCN possess local axonal terminals. In my impregnations, the majority of cells in which axons were impregnated maintained local projections. It is a difficult question as to whether the local terminals are collaterals from long axons that project outside the nucleus, or represent the entire axon of a local circuit neuron. In many cells that maintained local boutons, longer projections were not found. Two possible explanations are that a large number of cells in the SCN are local circuit neurons, or alternatively, that given the small caliber of SCN axons, the Golgi nera reazione did not successfully impregnate the entire axon in which case the probability would be that longer axons, or long axons of cells with local collaterals, would be less likely to be found. Relevant to this question is the report of Szentágothai et al., ('72) that with their Golgi impregnations they did not see any local collaterals arising from longer projection axons of SCN cells. This would lend credence to the hypothesis that, in terms of axons, there exist three populations of cells: those with only local projections, those with only long projection axons, and those with local collaterals and long projection axons. On the basis of ADH-neurophysin-positive immunocytochemistry, SCN projections to at least ten loci, intra- and extrahypothalamic, have been postulated (Sofroniew and Weindl, '78; Buijs, '78; Buijs et al., '78); it seems unlikely that those ten loci would receive projections from ten different groups of SCN cells. One may hypothesize that one SCN neuron may possess quite extensive long axonal projections to a wide number of neural loci in the hypothalamus, thalamus, midbrain, and medulla. It is important to note, however, that a large number of cells in the SCN do
communicate with other SCN neurons with either short axons or local collaterals of long axons.

Another potential form of communication that could exist between SCN cells is through ephaptic interaction. An ephaptic interaction might occur in areas where cells are tightly packed together, and where large areas of membrane apposition occur, but in the absence of further membrane specialization (i.e., gap junction). The cells in the dorsomedial part of SCN would be hypothalamic candidates for ephaptic interaction. They are close together with long regions of membrane apposition separated by the usual 20 nm intercellular cleft. Based on light and electron microscopy, regions of neuronal apposition exceeding 25 \( \mu \)m are common in SCN. The space between the apposing membranes varies between 140A and 220A; this variability may be due to different fixatives, block and section stains, plane of sectioning and normal tissue variation.

Ephaptic interaction might take two forms. First, where an extended region of membrane apposition occurs, where membrane resistance is low, and where the lateral regions are insulated with a glial process, an action potential in one cell may directly elicit an action potential in the apposed neuron (Bennett and Auerbach, '69; Bennett, '72; Martin and Pilar, '64; Hess, '65). A second possibility is that while an action potential in the first cell may not directly cause the second cell to fire, with an alteration in the \( K^+ \) in the extracellular space of the apposition or direct current flow, the probability of the second cell's firing may be altered. Similarly, the release of neurotransmitter at a single SCN synapse would not fire the postsynaptic cell but would act in concert with other terminals to influence the polarization or depolarization of the postsynaptic cell. Potassium movement extracellularly from one neuron may influence potentials of both nearby neurons and glia (Grossman et al., '69; Baylor and Nicholls, '69). Ephaptic interaction between apposed dentate granule cell somata has been proposed (Laatsch and Cowan, '66). In their theoretical study on the olfactory bulb, Rall and Shepherd ('68) note that "... radial potential gradient generated by the granule cell population must have a direct electrical (ephaptic) effect on the mitral cell." This effect would have a hyperpolarizing effect on mitral cell bodies and a depolarizing effect on mitral dendritic terminals. This ephaptic interaction then would exert a slight inhibitory influence on the impulse trigger zone near the mitral axon hillock. While our understanding of the electrophysiology of SCN lags far behind our knowledge of the olfactory bulb, inhibitory ephaptic influences may also exist in the SCN.

Chains of cells, found commonly in the dorsomedial SCN, are unusual in other parts of the brain. In addition to playing a role in ephaptic interaction as described above, the occurrence of neurons in chains would increase the probability that extracellular current, ions, or neurotransmitters might spread from one neuron to influence nearby cells.

It is interesting that soma-soma appositions are common in the magnocellular region of the supraoptic and paraventricular nucleus. Ephaptic communication between hypothalamic magnocellular neurons in the supraoptic and circularis nuclei has been postulated (Lafarga et al., '75; Tweedle and Hatton, '76; '77). Neurons of these nuclei synthesize and secrete antidiuretic hormone (ADH). Similarly, the region of SCN that has the greatest frequency of soma-soma appositions is also the region that has the highest density of ADH-positive cells. If further studies find immunocytochemically that ADH-positive neurons of SCN are those with the cell appositions, this would indicate that perikaryal contacts may be a common characteristic of both magnocellular and parvocellular ADH-producing neurons. In support of this hypothesis, a recent EM immunohistochemical paper (van Leeuwen et al., '78) showed two SCN vasopressin-positive neurons in close proximity.

In addition to local interaction between cells within each suprachiasmatic nucleus, the left and right SCN can communicate with one another via axons crossing the midline, seen in the present study with Golgi impregnations, HRP filling, and certain silver stains.

Only a few cases were seen in Golgi impregnations where a short axon-like process contacted dendrites of the cell of process origin. Contacts such as these have been suggested in a number of other non-hypothalamic loci, including the cerebral cortex, cerebellum, brain stem, and spinal cord (Scheibel and Scheibel, '71; Shkolnik-Yarros, '71; Van der Loos and Glaser, '72; Chan-Palay, '71) and have been termed "autapses" (Van der Loos and Glaser, '72) or "autocellular collaterals" (Held, 1897). Millhouse ('73) reported autaptic-recurrent axon collaterals in the ventromedial hypothalamic nucleus. Autapses have been postulated to be involved primarily in recurrent inhibition, whereby "part of the neuron's input is put under the influence of the cell's own output . . ." (Van der Loos, '76); a self-excitatory func-
tion cannot yet be excluded. However, the presence of such suggestive contacts with the Golgi method does not constitute proof of actual synaptic contact; furthermore, even with ultrastructural substantiation, these morphological cases of autapses may be physiologically silent (Wall, '77).

Another type of self-innervation has been reported in the SCN by Guldner and Wolff ('78). They found with serial EM sections one case in which a dendritic spine made an apparent synapse onto the same dendrite from which the spine originated. In the present study, spinodendritic contacts are seen frequently in Golgi impregnations in the SCN of both young and adult rats and in the magnocellular paraventricular nucleus in young rats. No EM evidence confirming that these contacts were of synaptic nature was found in the present study. This type of self-innervation is unorthodox, and potentially functionally interesting, but requires further substantiation.

Combining the observations of the present study with the previous work of Guldner ('76; Guldner and Wolff, '74), most cells in SCN probably do communicate with one another through numerous local axons as well as dendro-dendritic, dendro-somatic, somato-somatic, somato-dendritic, and perhaps with ephaptic interaction between the tightly packed cells of the dorsomedial SCN.

Glia

The functional role of neuroglia in SCN should not be neglected. In the chains of neurons occurring predominantly in the dorsomedial SCN, astroglia are commonly found interspersed with the neurons. Ultrastructurally, some astroglia in SCN have a much richer component of intracellular organelles, especially Golgi apparatus and ribosomes, than are usually described in other neuronal loci. In fact astrocytes in SCN often have a richer organelle population than do nearby neurons (for instance, compare the rich cytoplasm of the astrocyte with the organelle-poor neuron 1 in Figure 4). Given the cytological details of astroglia in SCN, these cells appear to be well-equipped for synthetic activity; what is being synthesized and why is currently unknown. Long processes of astroglia reach from cell bodies deep in the optic chiasm and branch within the SCN in the area innervated by retinal axons. Furthermore, as in other areas of the brain, gap junctions exist between astroglia; gap junctions may serve to couple cells electrically, to couple cells metabolically, or to allow for intercellular passage of substances from one cell to another (Gilula et al., '72; Bennett, '72). The possibility of morphological and physiological interaction between astrocytes and neurons in SCN deserves further examination. Tweedle and Hatton ('76, '77) have reported a statistically significant increase in occurrence and length of somatic appositions in magnocellular neurons of the rat's hypothalamus as a result of dehydration; they suggest this effect is caused by a retraction of glial processes between perikarya. Surprisingly, this retraction could be detected after only 12 hours of water deprivation, suggesting that glial insulation and isolation is a dynamic rather than static phenomenon. They postulated that the glial retraction might serve to facilitate ephaptic interaction between cells responding to the dehydration.

In addition to acting as an insulation between nerve cells, glia may serve other roles such as participation in uptake of neurotransmitter and involvement in local electrolyte balances. Considerable attention has been given to the function of glia as a potassium sink, whereby the glia may work in conjunction with neurons in reducing extracellular potassium after neuronal action potentials (Varon and Somjen, '79, review).

General discussion

One pervasive theme that has, unfortunately, become embedded in some recent thinking is that the medial hypothalamus is "unorganized." Given the precise nature of the control of behavioral, physiological, and endocrine events (e.g., food intake, weight regulation, temperature control, circadian rhythms, hormone feedback, etc.), the organization of the medial hypothalamus must be more than a haphazard web of connections between cells. Combinations of older methods including Golgi impregnations and electron microscopy with recent advances in immunohistochemistry will undoubtedly reduce the apparent anatomical entropy in the hypothalamus.

The suprachiasmatic nucleus shows circadian variations in 2-deoxyglucose uptake (Schwartz and Gainer, '77), serotonin uptake (Meyer and Quay, '76), and amino acid uptake (van den Pol, in preparation). These variations may be endogenous or may be due to afferent axons terminating within SCN. If the SCN is involved in some capacity as a central circadian oscillator, the question remains as to whether the individual cell within SCN manifests an intrinsic circadian rhythm, or whether the
rhythm is a result of a complex patterning of ultradian oscillations (Nishino et al., '76). Either way, the neurons of SCN must take advantage of the many avenues of intranuclear communication either to synchronize independent oscillations or to allow synthesis of a circadian rhythm from shorter periods. The capacity of cells to maintain endogenous measurable circadian rhythms is not unusual; such rhythms have been described in unicellular organisms such as Paramecium (Ehret, '59) and Gonialax (Sweeney, '60), in plants (de Marain, 1722; Duhamel, 1758; Bünning, '36), and in vitro animal tissue including the adrenal gland (Andrews, '71) and Aplysia retina (Jacklet and Geronimo, '71). Cells of the SCN are not demonstrably different anatomically, and probably no different physiologically from other neurons or cells, at least in terms of the capacity for circadian rhythms. What sets neurons of the suprachiasmatic nucleus apart from neurons of other central nervous system loci is the interaction between cells in the nucleus and the nature of the efferent signals to other parts of the brain which in turn may or may not have their own endogenous cyclicity.

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SUPRACHIASMATIC NUCLEUS INTRINSIC ANATOMY


