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Suprachiasmatic Region of the Human Hypothalamus: Homolog to the Primate Circadian Pacemaker?

*Ralph Lydic, †William C. Schoene, *Charles A. Czeisler, and *Martin C. Moore-Ede

*Departments of *Physiology and †Pathology, Harvard Medical School, and †Department of Pathology (Neuropathology Division), Peter Bent Brigham Hospital, Boston, Massachusetts*

Summary: The suprachiasmatic nuclei (SCN) of the hypothalamus in mammals, including nonhuman primates, contain a key pacemaker of the circadian timing system. Examination of the histology of the anterior hypothalamus in human fetal, child, and adult brains indicates that there is a cluster of neurons which may be homologous to SCN. These neurons are more diffusely organized and laterally placed in human brains than is the SCN of nonhuman primates. **Key Words:** Circadian rhythms—Suprachiasmatic nuclei—Human hypothalamus—Primates—Circadian pacemaker.

In 1972, the suprachiasmatic nuclei (SCN) of the anterior hypothalamus were first identified in rodents as a putative pacemaker of the circadian (approximately 24 hr) rhythms which are displayed by many physiological and behavioral variables (Rusak and Zucker, 1979; Moore, 1979). The phase control which the 24 hr light–dark cycle exerts on these circadian rhythms is mediated by a retinohypothalamic tract (RHT) which originates in the retina and monosynaptically innervates the SCN (Moore, 1979). Since then a large number of studies have confirmed the central role which the SCN play in coordinating mammalian circadian rhythms (Rusak and Zucker, 1979).

Recently we have extended studies of SCN structure (Lydic and Moore-Ede, 1980) and function (Moore-Ede et al., 1980) to the squirrel monkey (*Saimiri sciureus*). Ablation of the SCN eliminates circadian rhythmicity in activity, feeding, and drinking, whereas other circadian rhythms such as body temperature persist. Thus, the SCN act as one of the key pacemakers in the multioscillator circadian timing system of this diurnal primate.

In humans, physiological studies have demonstrated that many important func-

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Address reprint requests to Department of Physiology, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115.

tions are temporally organized by the circadian system. Human subjects isolated from environmental time cues show circadian rhythms which free-run with a cycle length that differs from 24 hr, like those of other mammals (Czeisler, 1978; Weitzman et al., 1979; Wever, 1979). Clinical interest in these phenomena has been promoted by the observation that a sleep disorder, delayed sleep phase insomnia (Czeisler et al., 1979), and certain manic–depressive conditions (Wehr et al., 1979) can be treated by manipulating the circadian system.

Although the SCN have been consistently observed in nonhuman primates (Papez and Aronson, 1934; Moore, 1979; Lydic and Moore-Ede, 1980), their histological demonstration in man (Spiegel and Zweig, 1919; Kuhlenbeck and Haymaker, 1949; Wahren, 1959) has been elusive and even questioned (Clark, 1938; Ingram, 1940; Defendini and Zimmerman, 1978). However, because of the demonstrated importance of the SCN in controlling nonhuman primate circadian rhythms and the similarity between circadian rhythms in humans and nonhuman primates, we have undertaken anatomical studies of the suprachiasmatic region of the human hypothalamus.

METHODS

Initially, 6 human brains (age range, 28 weeks gestation to 16 years) from the Yakovlev Collection of human central nervous system material at the Armed Forces Institute of Pathology (AFIP) in Washington, D.C., were used to characterize the suprachiasmatic region of the hypothalamus. These brains (4 coronal, 1 sagittal, and 1 horizontal) had been embedded in celloidin, and serially cut into sections of about 35 μm. Every 20th section had been stained alternately with cresyl violet (CV) or Loyez stain. Generally, only every 40th, although in one case every 20th, section was available for study. An additional 10 human brains (age range, 4 months to 50 years), prepared in a similar manner, were selected at random for study from the portion of this Collection which is housed at the Boston City Hospital. One of these cases (MU 87-65) had also been studied at the AFIP, allowing study of every 20th section. We also have serially sectioned the hypothalami from 2 human brains (aged 25 and 48 years) at 15 μm thicknesses from paraffin-embedded material. Consecutive coronal sections from 1 hypothalamus were stained with cresyl violet (CV) and an occasional section stained with hematoxylin–eosin (H&E). The other hypothalamus was divided: one-half was serial-sectioned in the coronal plane and all sections stained as follows: every 10th section with H&E; the next section with Bielschowsky's silver method; the next with phosphotungstic acid hematoxylin (PTAH); and the remaining sections in this 10-section sequence with CV. The other half of this same hypothalamus was sectioned in the sagittal plane and all sections were stained as follows: every 10th section with H&E; the next section with luxol fast blue periodic acid Schiff (LFB-PAS); the next with Klüver-Barrera; the next section alternately with Bielschowsky's silver method and PTAH; and the remaining sections in the sequence with CV. For comparative purposes, we also prepared 6 squirrel monkey brains by cutting 50 μm thick frozen sections from tissue blocks of hypothalamus (Lydic and Moore-Ede, 1980). Every consecutive section was

saved and stained with CV. In addition, 2 Old World primate brains were studied from the Yakovlev Collection housed at the AFIP.

RESULTS

Figure 1 illustrates coronal sections from the suprachiasmatic region of New World primate, Old World primate, and human brains. In the squirrel monkey (Fig. 1A), the SCN are clearly visible, whereas in the *Macaca mulatta* (Fig. 1B), the SCN are less compact. Examination of a human fetus (Fig. 1C) revealed a cluster of cells in a location which generally corresponds to the vicinity of the SCN in New World (Fig. 1A) and Old World (Fig. 1B) primates. In Fig. 2, comprising entirely human material embedded in celloidin, the suprachiasmatic region is illustrated in three different planes of transection: horizontal-oblique (2A), coronal (2B), and sagittal (2C), and each shows a similar cluster of cells above the chiasm lateral to ventricle III.

The brains we used from the Yakovlev Collection at the AFIP were selected for study because each one contained a suggestive cluster of cells which could be seen in the suprachiasmatic region. However, because the 35 μm sections which were available from this Collection represent every 40th section (or, in one case, every 20th section), there was a gap of either 0.7 or 1.4 mm between successive sections. Brains randomly selected from that part of the Collection housed at the Boston City Hospital did not always reveal the suprachiasmatic cluster of cells, presumably because of this gap. When we systematically examined the frequency of observing a suprachiasmatic cluster of these cells in the second series of 10 brains selected at random from the Collection, such a cluster was only visible in 5 out of the 10 brains.

However, when we studied consecutive serial sections in coronal and sagittal planes cut at 15 μm intervals from paraffin-embedded material, each brain showed a small, poorly defined group of cells corresponding in location to those we have described in the celloidin material, i.e., above the chiasm and lateral to the floor of the optic recess of ventricle III. The special stains we used suggest that these cells we have identified in the suprachiasmatic region were neurons.

DISCUSSION

Our findings raise the question of whether the neurons we have identified in the suprachiasmatic region of humans are homologous to the primate SCN. It now seems appropriate to reexamine the long-standing controversy (Clark, 1938; Ingram, 1940; Defendini and Zimmerman, 1978) over the existence of the SCN in humans. This controversy appears to have been caused by several factors. First, the SCN have been described by at least 12 different terms since 1888, resulting in confusion over anatomical nomenclature even in species where the SCN are clearly visualized. For example, because of its shape in rodents, SCN were also referred to as *nucleus ovoideus;* however, we have recently demonstrated in squirrel monkeys that the SCN are far from ovoid (Lydic and Moore-Ede, 1980). Second, in humans there are significant phylogenetic changes in the shape of the

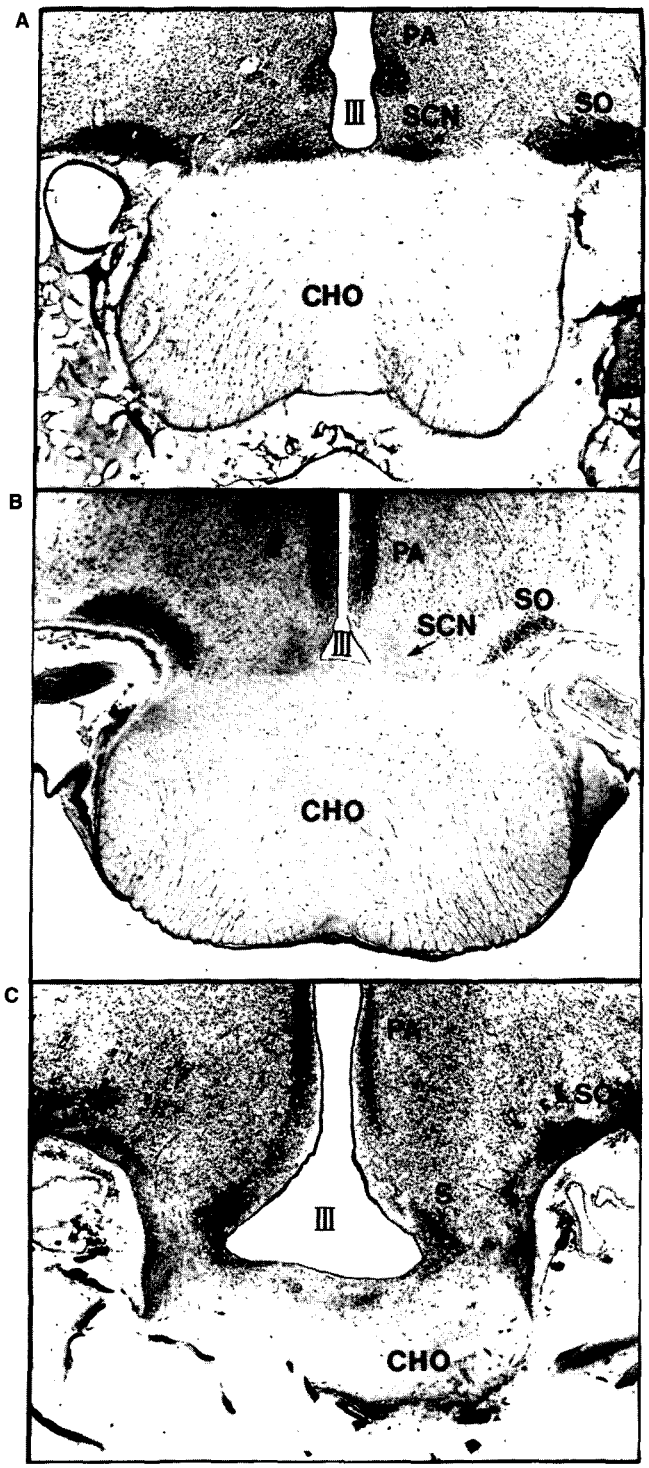

FIG. 1. Photomicrographs of cresyl violet-stained serial sections (15×). A: Coronal section through the anterior hypothalamus of the squirrel monkey showing suprachiasmatic nuclei (SCN) situated above the optic chiasm (CHO) and bilateral to the optic recess of ventricle III. B: Coronal section showing the more diffuse cellular grouping of the SCN in *Macaca mulatta* and the optic recess of ventricle III. C: Coronal section of the suprachiasmatic region of the male human fetal hypothalamus (28 weeks gestation; W166-64). A cluster of neurons (S) can be observed at either side of the optic recess of ventricle III. Paraventricular (PA) and supraoptic (SO) nuclei are also labeled.

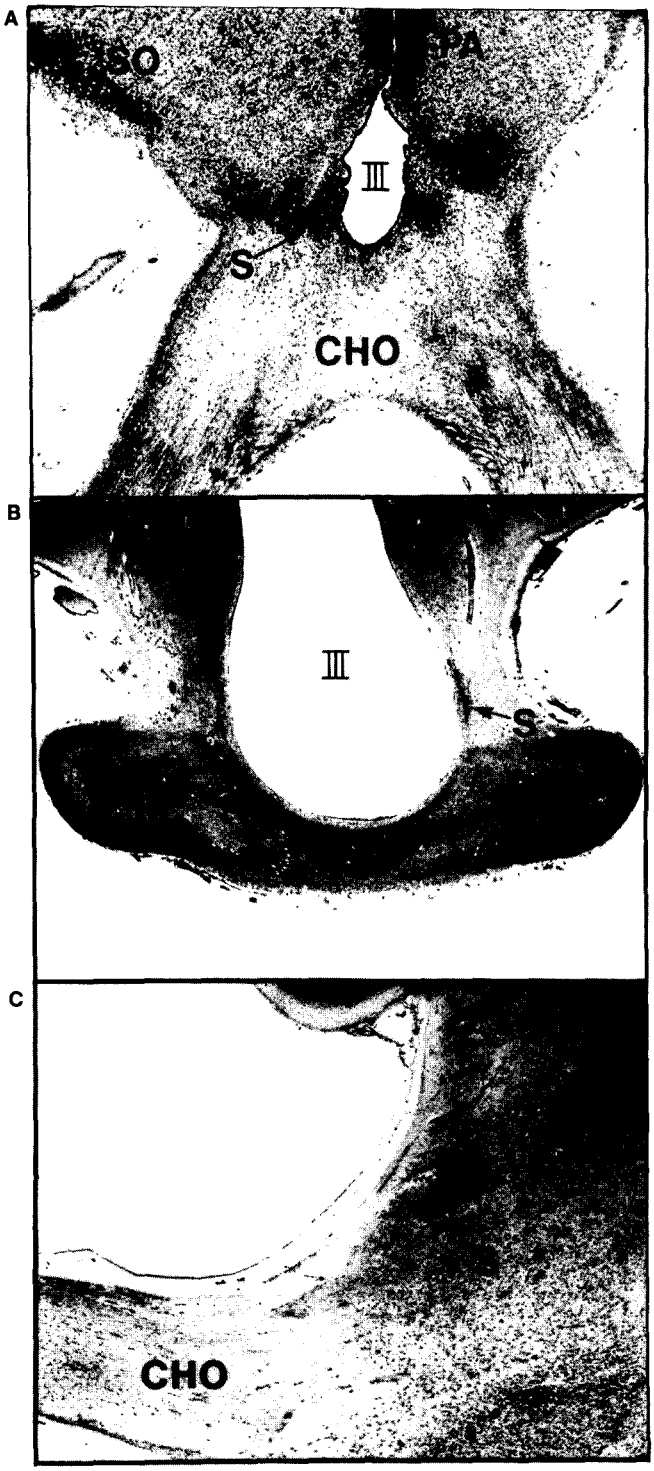

FIG. 2. Photomicrographs of cresyl violet-stained serial sections (15×). **A**: Horizontal-oblique section through suprachiasmatic region of female human hypothalamus (fetus 28 weeks gestation; B279-62) showing cluster of neurons (S) at each side of ventricle III. Optic nerves converging to form optic chiasm (CHO) are visible. **B**: Coronal section through 3.5-year-old male human hypothalamus (MU 89-65) showing neuronal cluster (S) in suprachiasmatic region on either side of ventricle III. **C**: Sagittal section through 16-year-old female human suprachiasmatic region (MU 87-65) showing cluster of neurons (S) dorsal to optic chiasm (CHO).

optic recess of ventricle III (Fig. 1) which have contributed to problems with the identification of the suprachiasmatic neuronal cluster in man. Third, the functional significance of the SCN in mammals has just been recognized (Rusak and Zucker, 1979), and interest in undertaking systematic structure–function correlations of the SCN in primates has only recently developed (Lydic and Moore-Ede, 1980; Moore-Ede et al., 1980). It also has not been ethically feasible to demonstrate an RHT in man by standard autoradiographic techniques. Furthermore, studies of the human anterior hypothalamus by consecutive serial sections are rare, and the practice of studying only intermittent sections may have precluded earlier identification of the human SCN.

In conclusion, we report there is a small, loosely packed cluster of neurons in the suprachiasmatic region of the human brain which may be homologous (Remane, 1961) to the nonhuman primate SCN. This neuronal cluster is small in size and diffuse, and it is easy to see how it could have been overlooked, resulting in the controversy concerning the existence of SCN in man. Clearly, however, additional work needs to be done to verify and characterize the histology of the human SCN.

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