From *Suprachiasmatic Nucleus: The Mind's Clock*
Proceedings of the NICHD Conference
Oct. 10-12, 1989, Bethesda, MD
D.C. Klein, R.Y. Moore and S.M. Reppert, Eds.
1991, Oxford University Press, New York, NY

Part III
INTRODUCTION

MARTHA U. GILLETTE

INTRINSIC SCN RHYTHMS

The ubiquity in eukaryotes of temporal programs expressed as circadian rhythms raises the central question: How is that temporal program written? (Pittendrigh 1989.) As Pittendrigh observed in his introductory remarks, this question has two parts. The first relates to understanding the substrates of the temporal program, the mechanism underlying a biological clock that keeps near 24-hour time. The second relates to understanding how the central pacemaker dictates the temporal program. What language does it use? How does it specify both spatially differentiated output lines (anatomically limited signals) and temporally differentiated output lines (messages specific to limited portions of the circadian cycle)?

Addressing this fundamental question requires first the identification of a central circadian pacemaker. The current intense interest in the workings of the suprachiasmatic nuclei (SCN) is based upon the multitude of studies that by specific lesion, transsection, and transplantation together established the paired SCN as a critical locus for entrainment and organization of behaviors, physiology, and metabolism into daily rhythms. The concordance of these data emphasizes the importance of determining the intrinsic functional organization of the SCN and of directly probing its temporal program. The question considered above now can be reformulated in terms of the identified neural substrate. How does this aggregate of tiny neurons and glia, through intrinsic biochemical, molecular, and ionic processes as well as cellular interactions, generate, regulate, and relay temporal information?

From the finding that the suprachiasmatic nuclei with intact efferent connections are necessary to organize behaviors into daily rhythmic patterns, it follows that there must be functions within the SCN that vary rhythmically. These intrinsic SCN rhythms might occupy two types of functional positions that are analogous to the two-part question posed earlier. One, they might be part of the timekeeping mechanism. In this case they would vary as they played their roles in temporal procession; an alteration in their levels of activity at another time would induce resetting of the temporal program. Two, they might be dictated by the pacemaker and act as messages relaying temporal information to other SCN cells,

brain regions, or body tissues. These roles might not be mutually exclusive, should an output signal feed back upon the pacemaker mechanism.

The chapters in Part III analyze data pertaining to intrinsic SCN rhythms. Primary SCN circadian rhythms identified in vivo include spontaneous neuronal electrical activity, glucose utilization, and vasopressin synthesis and secretion. That these represent intrinsic rhythms, generated within the SCN rather than driven by inputs, has been confirmed in each case through in vitro studies of SCN in brain slices or explants.

The first chapter of the four in Part III is mine; it addresses the intrinsic rhythm of spontaneous neuronal activity within the SCN. A circadian rhythm of multiunit activity was first described by Inouye and Kawamura (1979) in both intact and deafferented SCN in freely behaving animals. Because cutting SCN efferents abolished rhythms seen in intact brain regions, some aspects of the neuronal activity rhythm must be pacemaker output lines responsible for coupling to subsidiary oscillators. However, it is very possible that some aspects of this rhythm, such as membrane potential itself, are elements of the pacemaker. This chapter focuses on (1) characterization of the electrical activity rhythm of the ensemble of SCN neurons in brain slices (Prosser and Gillette 1989), (2) the functional organization of the pacemaker within the SCN, and (3) evidence for temporally limited windows of SCN sensitivity to stimulation of different transduction pathways. Because these periods of changing sensitivity are observed in vitro in surgically isolated SCN, the changing sensitive periods themselves are intrinsic SCN rhythms.

Chapters 7 and 8 by Schwartz and Newman, respectively, consider different aspects of intrinsic SCN metabolic rhythms. Diurnal variation in glucose utilization, measured by ^{14}C-2 deoxyglucose uptake in vivo, was the first intra-SCN rhythm reported (Schwartz and Gainer 1977). Energy metabolism is unusually high in the SCN compared with other brain regions. Schwartz considers the possible sources of these high energy demands relative to SCN functional activity. Newman's chapter evaluates the quantitative study of glucose utilization in SCN in vitro; he identifies three possible components of SCN glucose utilization. With this well-defined system, the relative contribution of various cellular activities to the metabolic rhythm can be dissected (Newman et al. 1989). The relationship(s) of metabolic rhythms to the pacemaker versus output temporal programs is unknown.

The last chapter in this section—Chapter 9 by Majzoub—addresses the only identified gene product that is regulated in a circadian fashion within the SCN: the messenger RNA for vasopressin. As vasopressin is released rhythmically for several days from SCN in vitro (Earnest and Sladek 1986, Gillette and Reppert 1987), this circadian rhythm of transcription must be regulated by the SCN pacemaker. Because previous work suggests that vasopressin injected into the SCN does not reset behavioral rhythms (Albers et al. 1984), regulation of vasopressin mRNA must lie on an output pathway. This affords the opportunity to examine

the detailed interaction between the SCN pacemaker and the temporally limited expression of the prepropressin gene (Majzoub et al. 1987).

The first observation of an intrinsic SCN rhythm was made in 1977, and most of the data discussed in this section is very recent indeed. Thus, we are only just beginning to identify and understand intrinsic rhythmic properties of the SCN. The accessibility and robustness of intrinsic rhythms have been surprising. The opportunity to use these rhythms to probe pacemaker mechanisms that might underlie regulation of time keeping is at hand. These studies inevitably raise the issue of the functional position of the intrinsic rhythm within the SCN—is it part of the pacemaker or of an output? This has not been an easy question to address because of the heterogeneity of cell types within the SCN. Nevertheless, as methods of assay become more refined, these issues will become clear. In the meantime, such studies contribute remarkably to deciphering the SCN's central temporal program.

REFERENCES

Albers, H.E.; Ferris, C.F.; Leeman, S.E.; Goldman, B.D. Science **223:**833–835; 1984.
Earnest, D.J.; Sladek, C.D. Brain Res. **382:**129–133; 1986.
Gillette, M.U.; Reppert, S.M. Brain Res. Bull. **19:**135–139; 1987.
Inouye, S.-I. T.; Kawamura, H. Proc. Natl. Acad. Sci. USA **76:**5962–5966; 1979.
Majzoub, J.A.; Carrazana, E.J.; Schulman, J.S.; Baker, K.J.; Emanuel, R.L. Am. J. Physiol. **252:**E637–E642; 1987.
Newman, G.C.; Hospod, F.E.; Patlak, C.S. J. Neurochem. **51:**1783–1796; 1989.
Pittendrigh, C. SCN: The mind's clock. NICHD meeting; 1989 October 10; Bethesda, MD.
Prosser, R.A.; Gillette, M.U. J. Neurosci. **9:**1973–1981; 1989.
Schwartz, W.J.; Gainer, H. Science **197:**1089–1091; 1977.