The Suprachiasmatic Nucleus: A 25-Year Retrospective

David R. Weaver[1]
Laboratory of Developmental Chronobiology, Pediatric Service, Massachusetts General Hospital, Boston, MA 02114, and Department of Pediatrics, Harvard Medical School

Abstract The suprachiasmatic nuclei (SCN) of the anterior hypothalamus contain the master circadian pacemaker in mammals. On the occasion of the 25th anniversary of the discovery of the SCN as the circadian clock, Charles A. Czeisler and Steven M. Reppert organized a meeting to review milestones and recent developments in the study of the SCN. The discovery that the SCN contain tissue necessary for generation of circadian rhythmicity was established by lesion studies published in 1972. The second phase of study demonstrated unequivocally that the SCN contain an autonomous circadian pacemaker. The principal studies in this period showed the presence of metabolic and electrical activity rhythms in the SCN in vivo and progressed to studies showing that the SCN maintain rhythmicity in vitro, demonstrating that the transplanted SCN can restore circadian function following destruction of the host SCN and ultimately showing that single SCN "clock cells" exhibit independent rhythms in firing rate. The third phase of study, aimed at identifying the biochemical and molecular mechanisms responsible for rhythmicity within the SCN, has begun with the identification of circadian mutants (*tau* mutant hamsters and *Clock* mutant mice) and the isolation of the *Clock* gene. This report traces the important steps forward in our understanding of the suprachiasmatic circadian clock by recounting the information presented at the SCN Silver Anniversary Celebration.

Key words suprachiasmatic nucleus, circadian rhythms, meeting report, historical article

Circadian rhythms are endogenous rhythms in physiology or behavior with a cycle length of approximately 1 day. The "circadian timing system" consists of input pathways that convey information to the circadian pacemaker, the circadian pacemaker itself, and the mechanism leading to expression of rhythmic outputs. Overwhelming evidence has accumulated over the past 25 years to show that the suprachiasmatic nuclei (SCN) of the anterior hypothalamus are the site of the master circadian pacemaker in mammals. The highlights in this period show that the SCN are necessary for circadian behavior, that the SCN are rhythmic in vivo, and that the SCN contain an autonomous circadian clock that maintains rhythmicity when isolated in vivo, when isolated in vitro, or when used in transplantation. Taking advantage of the fact that most roads to the 1997 Gordon Conference on Chronobiology (held in New London, New Hampshire) passed through Boston, Charles A. Czeisler and Steven M. Reppert organized a meeting to celebrate the 25th anniversary of the "discovery of the SCN as the circadian clock" and to review these milestones and recent developments in the study of the SCN. The meeting was held 8-9 August 1997 at Harvard Medical

1. To whom all correspondence should be addressed, at Laboratory of Developmental Chronobiology, GRJ 1226, Massachusetts General Hospital, Boston, MA 02114.

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 13 No. 2, April 1998 100-112

School and was attended by 135 scientists from around the world.

The format of the meeting was unusual in that speakers were encouraged to discuss how they came to make their contributions rather than just present their data. Speakers discussed the approaches, assumptions, insights, and errors that led to the articles forming the basis for our recognition of the SCN as the circadian clock in mammals (Table 1). However, recollection of the scientific basis for their discoveries was only a part of this meeting. The sometimes tortuous ways in which investigators came together were discussed, and the contributions of friends no longer among us (including Colin Pittendrigh and Gerard Groos) were recognized. Martha Gillette acknowledged the value of critical input from colleagues, and David Welsh listed on the blackboard a set of guidelines "for the younger investigators in the audience." Ironically, the previous speaker, Martha Vitaterna, had just listed advice she received from well-meaning colleagues that, if followed, would have led her to abandon the project that resulted in isolation and identification of the *Clock* mutation in mice. As always, the difficulty about advice is distinguishing the good from the bad; these presentations made it abundantly clear that these categories of advice will become distinct 5 years too late to be of value in the decision-making process. With the advantage of more than 5 years for most of these studies to prove themselves in the literature, the conference organizers had an easier job in identifying the studies that provided critical advances in understanding the SCN circadian clock. My objective in this meeting report is to recount the milestones as they were discussed by the speakers and to record some of the numerous anecdotes that were shared in the hopes of providing an enduring record of the SCN Silver Anniversary Meeting.

Table 1. List of speakers and highlighted references.

Session I: The Discovery	
Charles A. Czeisler, chair	Welcome and introduction
Robert Y. Moore	(Moore and Eichler, 1972)
Friedrich K. Stephan	(Stephan and Zucker, 1972a)
Irving Zucker	(Stephan and Zucker, 1972a)
Session II: Milestones	
David C. Klein, chair	Welcome and introduction
Shin-Ichi Inouye	(Inouye and Kawamura, 1979)
Benjamin Rusak	(Rusak, 1977)
William J. Schwartz	(Schwartz and Gainer, 1977)
Martha U. Gillette	(Prosser and Gillette, 1989)
Steven M. Reppert	(Reppert and Schwartz, 1983)
Fred W. Turek	After-dinner speaker
Session III: Recent Developments	
Steven M. Reppert, chair	Welcome and introduction
Michael Lehman and Rae Silver	(Lehman et al., 1987)
Martin Ralph and Michael Menaker	(Ralph et al., 1990)
Martha Hotz Vitaterna and Joseph S. Takahashi	(Vitaterna et al., 1994)
David K. Welsh and Steven M. Reppert	(Welsh et al., 1995)
Robert Y. Moore	"Whither the SCN"

Discovery of the SCN as the Circadian Pacemaker in Rodents

The discovery that the SCN represent a major circadian pacemaker in rodents occurred simultaneously in two laboratories, one headed by Robert Y. Moore (then at the University of Chicago) and the other headed by Irving Zucker at the University of California, Berkeley.

Moore came to study the SCN indirectly as an outgrowth of his interests in monoamines. Moore performed early lesion studies demonstrating ascending monoamine pathways from the brain stem to the limbic system. This led to collaboration with Julius Axelrod, Dick Wurtman, and Sol Snyder to study pineal monoamine metabolism and work showing that visual pathways controlling pineal biosynthetic activity run through the median forebrain bundle (Axelrod et al., 1966; Moore et al., 1967, 1969; Wurtman et al., 1967a, 1967b). This stimulated interest in defining visual pathways, which Moore pursued using "new" methods for tract tracing. Following injection of ^{3}H-amino acids into the eye, a novel bilateral projection to the hypothalamus, the retinohypothalamic tract (RHT), was identified in rodents (Moore et al., 1971; Moore and Lenn, 1972) and subsequently in other mammals (Moore, 1973). Hendrickson et al. (1972) independently identified the RHT in rats.

Fred Stephan and Zucker also were interested in visual pathways to the hypothalamus but from the perspective of understanding how light and the circadian clock influenced neuroendocrine function (e.g., the induction of persistent estrus in rats by constant light and the circadian control of the luteinizing hormone surge). Studies by Stephan and Zucker (1972b), Moore and colleagues (Axelrod et al., 1966; Moore et al., 1967; Moore et al., 1969; Wurtman et al., 1967a, 1967b) and others (Critchlow, 1963) showed that perception of light by the circadian system was independent of the visual pathways responsible for visually guided behavior (e.g., the primary optic tracts). Curt Richter had attempted to localize the circadian

clock in an extensive series of lesion studies and reported a single rat with a lesion in the anterior hypothalamus that disrupted rhythmicity (Richter, 1965). Moore was unaware of the Richter studies. To Stephan, Richter's studies provided more an indication of where the clock was not than of where it was. Zucker had read Richter's article and come to believe that a site important for circadian rhythmicity was present in the anterior hypothalamus. Richter also had suggested that this site might be "near the ventromedial nucleus." Of course, Zucker, with his firm command of neuroanatomy, might not have realized that the ventromedial hypothalamus is not in the *anterior* hypothalamus (see below). Hints existed in the literature to suggest that the SCN might be involved in photoreception relevant to neuroendocrine systems. A direct RHT had been proposed earlier, but the data were inconclusive prior to the tract-tracing studies of Moore (Moore and Lenn, 1972; Moore, 1973) and Hendrickson et al. (1972).

Moore's finding of the RHT (Moore et al., 1971) stimulated both groups to focus on the SCN as a site in the visual pathway to the circadian pacemaker (Moore and Eichler, 1972; Stephan and Zucker, 1972a). Both groups had the expectation that the SCN might be a relay on the visual input to a central oscillator and thus that destruction of the SCN might lead to free-running rhythms. Instead, electrolytic lesion of the SCN produced arrhythmicity, suggesting that the SCN (or a nucleus near it) were a necessary component of the central circadian oscillator (Moore and Eichler, 1972; Stephan and Zucker, 1972a). Stephan performed additional lesion studies with electrodes at an angle from the midline to further convince himself that it was the SCN, rather than a nucleus dorsal to it, that was the site of the circadian clock. Once Moore and Eichler's (1972) article was published, Stephan felt more comfortable with his assertion that the SCN were the site of the pacemaker, comforted by the knowledge that if he was wrong, then at least he was in good company.

Moore and Eichler (1972) assessed adrenal corticosterone content 3 weeks after placement of SCN lesions or knife cuts. This method requires pooling data from a population of subjects. Stephan and Zucker (1972a), on the other hand, were able to record locomotor activity and drinking behavior from individual animals, thanks to the technical advance (loosely used) that they acquired with access to Esterline-Angus event recorders. It is much easier to distinguish loss of oscillatory function from other alternatives (such as desynchronization of individuals in a population) when the rhythms of individual animals are studied, and the Stephan and Zucker (1972a) article is thus the more compelling one of the two (not compelling enough, however, for publication in *Science*, to which it was initially submitted). It is worth noting the Zucker laboratory's reluctance to believe the results from initial experiments in which 3 animals with SCN lesions became arrhythmic, indicated by the heading on a page from Stephan's data book in which a replicate experiment had been dubbed "Fred's Folly II" by a laboratory assistant. This was not folly, as we can now appreciate, but (together with Moore and Eichler's [1972] report) rather was the foundation for recognizing that the SCN are the primary pacemaker regulating circadian rhythms in mammals.

Zucker's involvement in a seminal contribution to the neuroanatomy of the mammalian circadian system is incongruous with his performance in neuroanatomy while a graduate student at the University of Chicago. He shared that his performance in neuroanatomy was so poor that one instructor suggested he consider leaving graduate school. That instructor was Moore. In their presentations at the Boston meeting, Zucker did not comment on the quality of Moore's lectures in the classes in Chicago, but Moore did concede that his opinion of Zucker's neuroanatomical expertise has improved.

Milestones

After these initial reports involving destruction of the SCN, the field gathered momentum slowly. As noted by David Klein in his introduction to the milestones section, among these articles were studies demonstrating that the 100-fold rhythm in serotonin *N*-acetyltransferase (NAT) activity in the pineal gland, and thus the large-amplitude melatonin rhythm, represents a reliable output of the circadian clock and is generated by the SCN (Moore and Klein, 1974; Klein and Moore, 1979). Klein also noted the wealth of current clinical research that employs melatonin measurement as a marker of the circadian clock in humans. From an early stage, studies of the mammalian SCN and of melatonin were coupled. Many researchers maintain interests in both the SCN and melatonin.

Refined Assessment of the Effects of Destruction of the SCN

Among the most notable of the lesion studies that followed the initial "discovery" studies is a study by

Benjamin Rusak conducted while he was a graduate student with Zucker at Berkeley (Rusak, 1977). An important aspect of this work was the use of Syrian hamsters (see also Stetson and Watson-Whitmyre, 1976), which have become the species of choice for many laboratories because of their remarkably precise circadian activity rhythms. Hamsters also are seasonal breeders, which allowed investigation of the circadian mechanisms underlying photoperiodism. Rusak also pioneered efforts in the mathematical analysis of rhythmicity, with the assistance of David Brillinger's time-series analysis, which allowed (most of) the field to pass from the "Rorschach" method of assessing rhythmicity to a more scientific and statistical approach. This careful analysis revealed additional periodicities, notably 8- and 12-h periodicities in lesioned hamsters, and led to the proposal that the SCN is a master integrator (composed of populations of oscillators) that coordinates the activity of a variety of independent oscillators. Rusak also described studies that required him to assess the extent of visual function remaining in hamsters with lesions. His intention was to train hamsters to use visual cues to select one door of a "choice paradigm" to avoid foot shock. The hamsters foiled the behavioral paradigm by scent marking to distinguish the safe area, jumping to avoid shock, and selecting both options at once in a choice paradigm. With considerable time and effort, Rusak finally succeeded in showing what he knew at the start, that an enucleated hamster was in fact blind. It appears that this stage of his academic career might have served us all by preparing him for the frustration he was to encounter as the first editor of the *Journal of Biological Rhythms* (1986-1994).

The SCN Oscillate in vivo: SCN Neurophysiology

The next stage in the appreciation of the pacemaker role of the SCN came from studies showing that the SCN oscillate in vivo. The Introduction section of the Inouye and Kawamura (1979) article, which Shin-Ichi Inouye credits to Pittendrigh, made it clear that definitive demonstration of the presence of an autonomous circadian clock in a tissue could be accomplished by one of two means—transfer of clock properties with tissue transplantation or demonstration of rhythmicity within the tissue in vitro. The studies that succeeded in realizing these goals represent a later wave of milestone studies.

Inouye described studies demonstrating that multiunitelectrical activity in the SCN is rhythmic, with higher levels during the day, and that this rhythmic electrical activity persists within "hypothalamic islands" containing the SCN (Inouye and Kawamura, 1979). In intact rats, the firing rate outside the SCN is higher at night. This rhythmicity is lost following isolation from the SCN. Of 51 rats whose data were presented in the article, the critical results came from 4 rats with complete isolation of the SCN within a hypothalamic island. These technically demanding studies demonstrated the autonomy of the SCN and suggested that the SCN imparts rhythmicity on other structures by way of its neural connections. (More recent evidence suggests that diffusible substances from the SCN also might play a role in driving rhythmic behavior [Silver et al., 1996]).

The SCN Oscillate in vivo: Metabolic Activity Measurement with ^{14}C-2-deoxyglucose

William J. Schwartz described studies of SCN metabolic activity, the value of being at the right place at the right time, and the importance of being well dressed. The place was the National Institutes of Health campus in Bethesda, Maryland, the time was the middle 1970s, and Schwartz was impeccably dressed (as always). One of his research subjects seemed to be particularly appreciative of his sense of fashion, based on the enthusiasm with which it greeted him. Schwartz, a neurologist, was initially involved in electrophysiological studies of the primate *Substantia nigra* under the direction of Ed Evarts but became involved with the "hot new method" of studying metabolic activity through use of radiolabeled 2-deoxyglucose (2-DG) pioneered by Sokoloff and colleagues (Schwartz et al., 1976). Application of the 2-DG method to the SCN allowed Schwartz and Gainer (1977) to demonstrate directly that the SCN oscillate in vivo. During the daytime, SCN metabolic activity is high, independent of environmental lighting. SCN metabolic activity is low at night but can be increased by acute exposure to light. Activation of the SCN by light at night was consistent with data showing that pineal NAT activity was shut off by light at night and that the pathway to the pineal passed through the SCN (Klein and Weller, 1972; Moore and Klein, 1974; Klein and Moore, 1979). Persistence of the SCN metabolic activity rhythm in the absence of environmental lighting cues demonstrated its endogenous origin, although it was many years until the autonomy of this rhythm was shown by studies of the SCN in vitro (Newman and Hospod, 1986). Schwartz and Gainer (1977) also suggested that the 2-DG method would be useful in assessing neural structures responsible for

generating circadian rhythms in vivo, providing an important complement to the only method used to that point—destruction of the SCN.

The SCN Contain an Autonomous Circadian Clock: in vitro Electrophysiology

The study of Inouye and Kawamura (1979) was pivotal in leading to studies of SCN neurophysiology using the in vitro hypothalamic slice preparation. Martha Gillette, of the University of Illinois, traced the development of this field, which began with the near-simultaneous appearance in 1982 of articles from three groups describing rhythms in the firing rate of SCN neurons in culture (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982). This approach produced one of two lines of evidence directly demonstrating the autonomy of the SCN circadian pacemaker (the other is transplantation).

The approach used by Dan Green and Rhanor Gillette most resembles the way in which this method is now used in that SCN slices were maintained in vitro for several hours and rhythmicity in the firing rate of the population was detected by sampling many single units over time (Green and Gillette, 1982). Green's movement out of Rhanor Gillette's laboratory, Martha Gillette's interest in second messengers based on her previous work on invertebrates, and her desire to find a niche outside her husband's research shadow led her to take up the SCN slice work. Martha Gillette has become the champion of this approach, performing many of the studies that establish the in vitro SCN slice as a valid method for studying responsiveness of the SCN to neurochemical inputs and also allowing studies of signal transduction pathways that are not feasible in vivo. In this method, the SCN are maintained within a 500-micron thick coronal slice in warm, oxygenated buffer containing salts and glucose, and the firing rate of single SCN neurons is sampled for up to three cycles under constant conditions in vitro. This previously unequaled ability to extend the recording into the second and third days allowed Rebecca Prosser and Martha Gillette to prove that the shifts in the time of peak firing rate observed in vitro reflect a stable phase shift of the underlying oscillator (Prosser and Gillette, 1989). Furthermore, this article reflected the proof-of-principle for the in vitro approach to resetting the circadian clock by demonstrating that manipulations that elevate cyclic AMP levels (or stimulate adenylyl cyclase) cause phase-dependent phase shifts of the electrical activity rhythm. This method has been used by many investigators in subsequent years, including Martha Gillette's laboratory, which continues to use the SCN slice to study neurochemical and second messenger pathways influencing the SCN circadian clock.

The Developing SCN Contain a Functional Circadian Clock

Reppert described experiments demonstrating that the circadian clock in the SCN is a functional, entrainable oscillator prior to birth. An early indication that this might be the case came from studies by Deguchi (1975), who showed that the level of NAT activity in rat pups reared in constant darkness since before birth was not random but rather was rhythmic. Direct demonstration of a functional clock mechanism in the fetal SCN required direct measurement of an intrinsic aspect of the SCN. The opportunity to address this came about when Schwartz came to Massachusetts General Hospital as a faculty member in the Neurology Service, bringing his expertise with the 2-DG method with him. Ignoring the fact that Fuchs and Moore (1980) had reported that there is no rhythm in metabolic activity in the fetal SCN, Reppert and Schwartz (1983) demonstrated that rhythms in metabolic activity are present in the fetal SCN; in the fetal SCN, high metabolic activity occurs during the day, as in the maternal SCN. Additional animal models have been used to demonstrate that entrainment of the fetus occurs in several species (Davis and Gorski, 1986; Reppert and Schwartz, 1984; Weaver and Reppert, 1987), but not in all species (Rivkees and Reppert, 1990). In rabbits, prenatal entrainment likely has a marked positive impact on postnatal growth and development (Hudson and Distel, 1989). More recent studies have focused on mechanisms of maternal entrainment. The maternal SCN are necessary for the entrainment of the fetus, and time-of-day information is communicated from mother to fetus by redundant signals (for review, see Reppert, 1995). Two "signals" that are capable of entraining the fetal SCN are dopamine and melatonin (Viswanathan et al., 1994; Viswanathan and Davis, 1997), and the investigation of cellular and molecular mechanisms underlying maternal entrainment of the fetus continues. Other important questions, such as what determines the potential of an SCN cell to function as a circadian oscillator, are just beginning to be addressed. The oscillation of metabolic activity within the fetal SCN cell is remarkable when one considers that metabolic activity generally reflects energy used for Na^+-K^+-ATPases involved in restoring ion gradients across the cell membrane (Schwartz et

al., 1979; Mata et al., 1980), but the fetal SCN cell is virtually devoid of synapses (Moore and Bernstein, 1989) and has extremely low levels of electrical activity (Shibata and Moore, 1987). How and when individual SCN neurons become rhythmic and synchronized to a single phase remains to be determined. Entrainment of the fetus is a unique example of nonphotic entrainment.

Localization of the Circadian Clock "Brought Respect" to the Study of Rhythms

Fred W. Turek presented an after-dinner address that was largely humorous and full of "Harvard bashing," aimed at the conference organizers. Turek described the unusual photophilia (spotlight-seeking behavior) that appears to go along with affiliation with Harvard Medical School. Moving on to science, Turek noted that one of the last proponents of the idea that circadian rhythms represented responses to unseen environmental perturbations (rather than an intrinsic timekeeping mechanism) was Frank Brown, then a professor of biology at Northwestern University. Turek noted the irony in having followed in these footsteps; he is now a professor of biology at Northwestern. Turek then provided an interesting perspective on the importance of the discovery that the SCN function as the circadian clock in mammals. In the 1970s, biological rhythms research was lumped (by some observers) in a disreputable category along with "biorhythms," astrology, and mood rings. Turek maintained that the discovery of a discrete brain area that regulated circadian rhythms "gave us respect" and put to rest proponents of Brown's viewpoint. As a result of this discovery, circadian rhythms became viewed not simply as endocrine or behavioral fluctuations but also as the output of a central circadian oscillator worthy of study. The discrete localization of function to a small area of brain attracted attention as a model for the neural basis of behavior. These factors, combined with the many levels at which circadian rhythms can be studied (from endocrine / physiological to anatomical, cellular, and molecular), continue to attract investigators with varied backgrounds to study the SCN.

Turek also traced the relationship between the study of the SCN and the study of pineal melatonin. In mammals, melatonin is important for seasonal reproduction (in some species) but has only a modest impact on the circadian system. By contrast, melatonin does not play a role in seasonal reproduction in birds but is very important—even critical—for circadian organization (in some species). Furthermore, the circadian rhythm of melatonin production has been one of the rhythms used most extensively as a marker, or output, of the circadian clock. These facts have coupled the study of circadian biology with that of melatonin, for better or for worse.

Recent Developments

In his introduction to the "Recent Developments" section, Reppert demonstrated that he does in fact know how to tie a necktie. Reppert explained that four papers were selected to represent recent advances in the study of the SCN and that the conference organizers intended that each paper would be discussed by the junior and senior members of each investigative team.

The SCN Contain an Autonomous Circadian Clock: SCN Transplantation

The organizational format described by Reppert was immediately ignored by Mike Lehman and Rae Silver in their discussion of SCN transplants (Lehman et al., 1987). Joined by collaborator Eric Bittman, the trio presented a memorable description of their work punctuated by songs with Lehman on guitar. Songs included *Treat Your Students Well* (referring to Marie Gibson's status first as a student of Silver's and later as the collaborator with the knowledge of how to perform hypothalamic transplantation), *Little Ticky Tock*, and *If I Were a Smart Man*. There were others, but I was laughing too hard to write them down. During one song, Marty Zatz walked down from his seat in the audience, tossed a few coins into the still open guitar case, turned, ascended a few steps, paused, shrugged in Jack Benny style, and returned to add a few more coins.

This very entertaining session included a description of initial attempts to make use of differences in period among strains of mice to show that period could be transplanted and the failure of this approach due to the poor precision of locomotor onset in mice and Bittman's difficulty in using the M4 bus to get uptown through a Manhattan traffic jam. The group eventually succeeded in using hamsters to demonstrate that transplantation of fetal hypothalamic tissue containing the SCN can restore behavioral rhythmicity in adults previously made arrhythmic by destruction of the SCN. The SCN transplant model has

drawn interest from those interested in clinical application of neural transplantation because there is a high rate of recovery (80-90%), a robust behavioral assay of function, and a good structure-function relationship. Indeed, it is the careful correlation between the anatomy of the grafts and the restoration of behavior (and not merely that this paper too was rejected by *Science*) that makes this article stand out among several demonstrating restoration of function following SCN transplantation. (Other articles resulting from transplantation work conducted during this period are DeCoursey and Buggy [1989], Drucker-Colin et al. [1984], and Sawaki et al. [1984].) More recent studies show that functional restoration can be accomplished using an SCN cell suspension (Silver et al., 1990), indicating that the structural integrity of the SCN need not be maintained (or perhaps that the SCN can re-form its own structural integrity following transplantation). The transplantation model makes readily apparent several critical questions, including the need to identify the efferent signals from the SCN (see Silver et al., 1996), to identify the target structures that control specific circadian behaviors, and to identify the pacemaker cells within the SCN.

The tau *Mutation*

Martin Ralph and Michael Menaker presented their recollection of studies leading to the discovery of the *tau* mutant hamster and its use in transplantation studies to unambiguously demonstrate that a major feature of the circadian clock, its free-running period, can be transferred by transplantation of SCN tissue. Ralph was involved in studies of chronopharmacology in Menaker's laboratory at the University of Oregon in 1985 (cf. Ralph and Menaker, 1985) when he noted that a Syrian hamster obtained from Charles River Laboratories had an extremely strange activity pattern, with much of the activity occurring during the day. The circadian cycle length (*tau*) of the animal in constant darkness was unusually short, and subsequent breeding indicated that the mutant short-period phenotype was semi-dominant and inherited in a Mendelian fashion (Ralph and Menaker, 1988). In contrast to normal hamsters (which have a free-running period of 24 h), heterozygous *tau*/+ mutant hamsters have a free-running period of approximately 22 h, whereas the homozygous *tau*/*tau* mutants have a period of approximately 20 h.

Transplantation of SCN tissue between hamsters of these genotypes demonstrated that the period of restored rhythmicity was characteristic of the donor SCN rather than of the host (Ralph et al., 1990). These studies represent a milestone because they brought to a close the phase of study begun with SCN lesions that identify the SCN as the major pacemaker in rodents. More recently, *tau* mutant hamsters have been used to demonstrate that a circadian clock exists in the hamster retina and that this clock is affected by the *tau* mutation, as is behavioral rhythmicity (Tosini and Menaker, 1996). Thus, the *tau* mutation affects circadian clock function rather than SCN function specifically.

Ralph, now at the University of Toronto, made several interesting points related to the *tau* mutation. If the *tau* mutation had occurred in a genetically tractable animal such as a mouse, the effort would have been to isolate the gene and clone it, and the discovery of the mutation would not have been consummated until the cloning was completed. The lack of molecular tools for genetic analysis of hamsters means that we, collectively, are stuck analyzing the phenotype, taking advantage of it for what it will reveal, without (many) being seduced into attempts to clone it. Nevertheless, discovery of the *tau* mutation in hamsters might have encouraged investigators to search for induced mutations in circadian clock function (Pickard et al., 1995; Sollars et al., 1996; Vitaterna et al., 1994). It is also worth noting that if this mutant had gone to a laboratory not involved in circadian studies, then the mutation would have been lost. Also noteworthy is that the founder hamster was stolen in a laboratory break-in; thus, this remarkable, irreplaceable mutant line would have been lost had offspring not been available with which to replenish the stock.

Molecular Genetics: Generation and Identification of the Clock *Mutation*

Identification of a mutation affecting the circadian system in mice, *Clock*, represents another milestone in chronobiology, as described by Vitaterna and Joe Takahashi of Northwestern (Vitaterna et al., 1994). Vitaterna came to Northwestern with an interest in behavioral genetics and did her doctoral work in Turek's laboratory, studying strain differences in hamster circadian rhythms (cf. Vitaterna and Turek, 1993). When Takahashi and Turek decided to attempt a forward genetic approach to mouse circadian rhythms, Vitaterna moved down the hall as a postdoctoral fellow to focus on a

mutagenesis screen for clock mutations, an effort that soon became the primary focus of the Takahashi laboratory.

The forward genetic approach of mutagenesis and screening of progeny has been remarkably successful in circadian biology. Induced mutations affecting clock function have been identified in *Drosophila melanogaster* (Konopka and Benzer, 1971; Sehgal et al., 1994), *Neurospora crassa* (Feldman and Hoyle, 1973; Crosthwaite et al., 1997), cyanobacteria (Kondo et al., 1994), and *Arabidopsis thaliana* (Millar et al., 1995). As described by Vitaterna, successful application of this method to mice disproved several common assumptions about forward genetics including the assumption that the rate of mutation is too low, the assumption that screening of heterozygotes will not reveal defects (in fact, many clock mutations are dominant or semidominant [Dunlap, 1990]), and the fear that any mutant animal identified will be so messed up as to preclude systematic analysis. The success of this project demonstrates that a forward genetic approach can be applied to complex behaviors in mammals and that, as Takahashi pointed out, it is more a numbers game than a fishing trip. As any fisherman knows, fishing is not always just luck but rather requires wisdom in choice of location, bait, and timing. As Vitaterna put it, the statement that "you guys were just incredibly lucky" is not true; although they might have been incredibly lucky to get the founder carrying the *Clock* mutation in the first shipment of 42 animals, they were not *just* incredibly lucky.

Heterozygous *Clock*/+ mutant animals have a longer than normal free-running period (Vitaterna et al., 1994). The homozygous mutants have a unique phenotype characterized by a very long period (approximately 28 h) in constant darkness, with eventual loss of rhythmicity after several weeks. The *Clock* mutation was localized to mouse chromosome 5, and the brute force effort of positional cloning began.

As described by Takahashi, the *Clock* locus recently has been cloned and the product, CLOCK, has been identified as a PAS-domain-containing member of the basic helix-loop-helix family of transcription factors (King et al., 1997; Antoch et al., 1997). The *Clock* mutation is a single A-to-T transversion in the intronic splice donor site in the intron just 3' of exon 19, which leads to deletion of exon 19 from the mRNA. Exon 19 encodes a portion of a glutamine-rich domain, which, by analogy to other proteins containing basic and helix-loop-helix domains, might represent a transcriptional activation domain. This suggests that the CLOCK mutant protein is a less than fully functional transcription factor. The structure also suggests that CLOCK might dimerize with other PAS-domain-containing nuclear proteins to form functional transcription factors. The recent cloning of mammalian genes with high homology to the PAS-containing *period* gene of *Drosophila* (Tei et al., 1997; Sun et al., 1997), together with the cloning of the *Clock* gene, has caused great excitement and the hope that the biochemical mechanisms (and perhaps even the molecules themselves) underlying circadian oscillations may be conserved across a broad range of species.

Oscillatory Properties of Single SCN Neurons

An enduring question in the study of the SCN has been, "What is the minimal functional unit capable of circadian rhythm generation?" Welsh came to this question with the bias that most, if not all, SCN cells would be capable of independent circadian oscillations. This bias led him to develop a novel approach, based on long-term recording of electrical activity from individual, dissociated SCN cells using a multimicroelectrode plate. The data from this project, conducted in the Reppert laboratory, show clearly that individual SCN neurons in culture can maintain independent free-running rhythms of firing rate (Welsh et al., 1995). Within a single culture, cells with distinct phases and periods can be detected with no evidence of oscillator interactions despite the clear evidence for functional synaptic interactions. The phenotypic identity of SCN neurons that possess oscillatory capacity remains to be determined, and it is not clear whether all cells displaying oscillatory capacity in vitro would represent functional oscillators in vivo. It also remains to be determined whether single SCN clock cells possess the capacity to phase shift and entrain and whether the phase response curve is a single-cell characteristic or a network phenomenon.

Welsh also recounted lessons about science and life learned along the way to his degrees. Trying something new can demystify exotic techniques and is a good experience to have early in one's career, and the Neurobiology course at the Marine Biology Laboratory in Woods Hole, Massachusetts, served that purpose well for Welsh. Welsh also learned the danger of meeting Society for Neuroscience abstract submission deadlines while having no data, and the need to think

before you talk (e.g., don't share your ideas publicly when you don't know how long it will be before you will be ready to go to press). Welsh described his apprehension at learning that "the $10 million Center for Biological Timing" was moving into "his" area while he was "one graduate student with a borrowed Macintosh." It is important to recognize that perception of your competition's interests and resources is likely inaccurate, that each individual brings to a problem his or her own unique approach and perspective, and that no area of research is "yours" until your work is published. Welsh also noted that tough problems require persistence and that persistence requires long-term support from one's mentor. In his brief remarks, Reppert noted that the idea and approach for this study originated with Welsh, that initial studies were carried out in Diomedes Logothetis's laboratory at Children's Hospital, and that the project came to fruition at Massachusetts General Hospital.

Whither the SCN

The meeting concluded with Moore providing his view of the direction of the field in a talk titled "Whither the SCN." I was relieved to learn that this was not a talk about pseudorabies infection of the SCN (Card et al., 1991), that is, wither the SCN.

Most of us conceptualize the circadian timing system as an input pathway, a central oscillator, and various output pathways. Moore predicts that we will make the most progress with the input pathway. This seems reasonable. The retina is the circadian photoreceptor in mammals, providing an unambiguous starting point, and the retinal ganglion cells projecting to the SCN have been defined. Evidence that glutamate is the transmitter of the RHT seems very strong. Although the circadian photoreceptor has spectral sensitivity similar to cones, not all present agreed with Moore that cones are *the* circadian photoreceptor. Moore proceeded to be even more provocative by stating that "there is no such thing as nonphotic entrainment." (Several of Nicholas Mrosovsky's hamsters have responded in kind, stating that there is no such thing as Bob Moore.) Moore's meaning was that nonphotic inputs should be studied in the context of their influence on photic entrainment, and indeed this is beginning (Ralph and Mrosovsky, 1992; Biello et al., 1997). I would suggest, however, that there is much to be learned from comparing the apparently distinct mechanisms by which the clock can be reset, for it is the diversity of mechanisms that will help identify the common denominators. Furthermore, entrainment of the fetus by maternal signals clearly represents a form of nonphotic entrainment (Reppert and Schwartz, 1983), and it occurs at developmental stages when photic entrainment is not possible (Speh and Moore, 1993).

Moving to discuss oscillator questions, Moore identified several issues that are important for understanding the role of the SCN as a circadian clock. These include determining where the anatomical borders between SCN and non-SCN cells occurs, determining whether all or only some SCN neurons possess oscillatory capacity, and understanding how the multiple oscillators within the SCN are coupled to produce a functional network pacemaker and determining whether specific subpopulations of oscillators are responsible for driving specific output rhythms. Moore suggested that internal desynchrony of rhythms might represent the dissociation of several functional SCN oscillator populations. Another issue is whether there are additional functional pacemakers. Clearly, the retina contains a circadian clock, there is a non-SCN food- entrainable oscillator, and there is some indication of an enteric pacemaker. Whether these different clocks are constructed of the same springs and gears remains to be determined. The issue of how alterations in gene products comprising the proposed molecular loop produce rhythmicity in action potentials (which appears to be the primary output of SCN neurons) also remains to be determined.

Turning to his anatomical slides, Moore presented evidence for a core and shell section of the SCN and noted the differences in connectivity of these regions. Core and shell represent new names, but the concept of a retino-recipient ventrolateral zone and a more dorsomedial zone has been well defined. It is Moore's opinion that "specialization of structure means specialization of function," but it is unclear what this diversity of cellular phenotypes and connectivity means in terms of the ability of the SCN to function as a pacemaker and communicate its message to target sites. Of the output pathways from the SCN, only the projection to the pineal gland is well established from start to finish. Moore posed the question of how other effector tissues receive information from the circadian

clock. Is it conducted by neural transmission or in some cases by volume transmission? Does firing rate alone convey information from the SCN to their targets?

Perspectives and Comments

The study of the SCN has advanced through distinct phases. In the initial phase, lesion studies led to the discovery that the SCN are necessary for most circadian rhythms in mammals (Moore and Eichler, 1972; Stephan and Zucker, 1972; Rusak, 1977). (One clear exception is the food-entrainable oscillator.) The second phase demonstrated that the SCN contain an autonomous circadian pacemaker. These studies began with metabolic and electrical activity measured in vivo (Schwartz and Gainer, 1977; Inouye and Kawamura, 1979), progressed to in vitro analysis (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982; Prosser and Gillette, 1989) including studies demonstrating that the transplanted SCN can restore circadian function (Lehman et al., 1987; Ralph et al., 1990), and ultimately to demonstrating that single SCN clock cells exhibit independent rhythms in firing rate (Welsh et al., 1995). The third phase, the search for the mechanisms for rhythmicity, has begun and will require understanding circadian mutants at the biochemical and molecular levels. The forward genetic approach being employed by the Northwestern group should continue to identify mutations in genes necessary for normal circadian behavior (Vitaterna et al., 1994; Takahashi et al., 1994). As we gain more information (or more precise hypotheses) about the molecular players that make up the tag team responsible for counting off 24 h, it will become possible to select interesting candidate genes and then disrupt them directly. Identification and study of genes necessary for normal circadian clock function, some of which might represent true clock genes, is among the highest priorities. A fourth stage, understanding how the many single-cell oscillators within the SCN coordinate their activity and interact to provide a unitary "circadian time" to the organism, remains to be addressed. The recent description of altered circadian function in mice that lack a form of neuronal cell adhesion molecule with polysialic acid may provide a starting point (Shen et al., 1997). To complement the reductionist approach to understanding the mechanisms underlying circadian clock function, it also will be important to study the functional relevance of the SCN (cf. DeCoursey et al., 1997).

Finally, it is interesting to note the cross-fertilization that has occurred between chronobiology and other fields in the past 25 years. Experimental approaches borrowed from other disciplines have contributed to the study of the SCN but also have given back considerably to those disciplines. The regulation of NAT activity in the pineal gland represented a useful model system for the study of monoaminergic neurotransmission; Axelrod received the Nobel Prize in 1970 for his pioneering work on aminergic neurotransmitter systems and the effects of drugs on them, a portion of which involved study of the pineal (Udenfriend, 1970). Application of the ^{14}C-2-DG method to study metabolic activity in the SCN helped to establish the presence of oscillations within the SCN in vivo but also was important in demonstrating the utility of this method for metabolic mapping, as noted by Sokoloff in receiving the Lasker Award. Methods for metabolic mapping of brain activity, including current noninvasive methods using PET and MRI, can trace their history back to the 2-DG method. Transplantation studies demonstrated that the SCN contain an autonomous circadian clock (Lehman et al., 1987) and that the SCN determine the cycle length (period) of circadian oscillation (Ralph et al., 1990), but they also contributed at an early stage to demonstrating the restoration of function possible through fetal tissue transplantation. Studies of photic induction of immediate early gene (IEG) expression in the SCN might have contributed to the snowballing of interest in IEGs in the 1980s (and represents one area in which a presentation was appropriate but was not included in the program; my choice would have been Kornhauser et al. [1990]). The successful isolation of the *Clock* mutation in mice (Vitaterna et al., 1994) demonstrated that the forward genetic approach is a viable method for identifying genes involved in complex behavior in mammals (Takahashi et al., 1994). Furthermore, Takahashi's group showed the utility of bacterial artificial chromosomes for transgenic rescue of a mutant phenotype in mice (Antoch et al., 1997). This is reminiscent of the pioneering success with transgenic rescue of arrhythmicity in per^0 flies by *p* element-mediated transformation (Bargiello et al., 1984), among the first studies showing that a complex behavior could be rescued by transgenesis. Thus, studies and approaches that have

made significant contributions in chronobiology also have resulted in significant contributions to the broader fields of neurobiology and physiology. It is worthwhile knowing the brief history of our field to recognize the methods we have borrowed from other fields, the contributions we have made to those other areas, and the diversity of individuals and approaches that have contributed to our understanding of the SCN as the master circadian pacemaker.

ACKNOWLEDGMENTS

I thank Steve Reppert and Chuck Czeisler for their efforts in organizing the meeting, Reppert for comments on an earlier draft of the article, and all the conference participants for their efforts in the past and for participating in the conference.

REFERENCES

Antoch MP, Song EJ, Chang AM, Vitaterna MH, Zhao Y, Wilsbacher LD, Sangoram AM, King DP, Pinto LH, and Takahashi JS (1997) Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. Cell 89:655-667.

Axelrod J, Snyder SH, Heller A, and Moore RY (1966) Light-induced changes in pineal hydroxyindole-*O*-methyltransferase: Abolition by lateral hypothalamic lesions. Science 154:898-899.

Bargiello TA, Jackson FR, and Young MW (1984) Restoration of circadian behavioural rhythms by gene transfer in *Drosophila*. Nature 312:752-754.

Biello SM, Golombek DA, and Harrington ME (1997) Neuropeptide Y and glutamate block each other's phase shifts in the suprachiasmatic nucleus in vitro. Neuroscience 77:1049-1057.

Card JP, Whealy ME, Robbins AK, Moore RY, and Enquist LW (1991) Two alpha-herpesvirus strains are transported differentially in the rodent visual system. Neuron 6:957-969.

Critchlow V (1963) The role of light in the neuroendocrine system. In *Advances in Neuroendocrinology*, AV Nalbandov, ed, pp 377-402, University of Illinois Press, Urbana.

Crosthwaite SK, Dunlap JC, and Loros JJ (1997) *Neurospora wc*-1 and *wc*-2: Transcription, photoresponses, and the origins of circadian rhythmicity. Science 276:763-769.

Davis FC and Gorski RA (1986) Development of hamster circadian rhythms. II: Prenatal entrainment of the pacemaker. J Biol Rhythms 1:77-89.

DeCoursey PJ and Buggy J (1989) Circadian rhythmicity after neural transplant to hamster third ventricle: Specificity of suprachiasmatic nuclei. Brain Res 500:263-275.

DeCoursey PJ, Krulas JR, Mele G, and Holley DC (1997) Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. Physiol Behav 62:1099-1108.

Deguchi T (1975) Ontogenesis of a biological clock for serotonin:acetyl coenzyme A *N*-acetyltransferase in pineal gland of rat. Proc Natl Acad Sci USA 72:2814-2818.

Drucker-Colin R, Aquilar-Roblero R, Garcia-Fernandez F, Fernandez-Cancino F, and Rattoni FB (1984) Fetal suprachiasmatic nucleus transplants: Diurnal rhythm recovery of lesioned rats. Brain Res 311:353-357.

Dunlap JC (1990) Closely watched clocks: Molecular analysis of circadian rhythms in *Neurospora* and *Drosophila*. Trends Genet 6:159-165.

Feldman JF and Hoyle MN (1973) Isolation of circadian clock mutants in *Neurospora crassa*. Genetics 75:605-613.

Fuchs JL and Moore RY (1980) Development of circadian rhythmicity and light responsiveness in the rat suprachiasmatic nucleus: A study using the 2-deoxy[1-^{14}C]glucose method. Proc Natl Acad Sci USA 77:1204-1208.

Green DJ and Gillette R (1982) Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic slice. Brain Res 245:283-288.

Groos G and Hendriks J (1982) Circadian rhythms in electrical discharge of rat suprachiasmatic neurones recorded in vitro. Neurosci Lett 34:283-288.

Hendrickson AE, Wagoner N, and Cowan WM (1972) An autoradiographic and electron microscopic study of retino-hypothalamic connections. Z Zellforsch 135:1-26.

Hudson R and Distel H (1989) Temporal pattern of suckling in rabbit pups: A model of circadian synchrony between mother and fetus. In *Development of Circadian Rhythmicity and Photoperiodism in Mammals*, SM Reppert, ed, pp 83-102, Perinatology Press, Ithaca, NY.

Inouye ST and Kawamura H (1979) Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. Proc Natl Acad Sci USA 76:5962-5966.

King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, Steeves TD, Vitaterna MH, Kornhauser JM, Lowrey PL, Turek FW, and Takahashi JS (1997) Positional cloning of the mouse circadian *Clock* gene. Cell 89:641-653.

Klein DC and Moore RY (1979) Pineal *N*-acetyltransferase and hydroxyindole-*O*-methyltransferase: Control by the retinohypothalamic tract and the suprachiasmatic nucleus. Brain Res 174:245-262.

Klein DC and Weller JL (1972) Rapid light-induced decrease in pineal serotonin *N*-acetyltransferase activity. Science 177:532-533.

Kondo T, Tsinoremas S, Golden SS, Johnson CH, Kutsuna S, and Ishiura M (1994) Circadian clock mutants of cyanobacteria. Science 266:1233-1236.

Konopka RJ and Benzer S (1971) Clock mutants of *Drosophila melanogaster*. Proc Natl Acad Sci USA 68:2112-2116.

Kornhauser JM, Nelson DE, Mayo KE, and Takahashi JS (1990) Photic and circadian regulation of c-*fos* gene expression in the hamster suprachiasmatic nucleus. Neuron 5:127-134.

Lehman MN, Silver R, Gladstone WR, Kahn RM, Gibson M, and Bittman EL (1987) Circadian rhythmicity restored by neural transplant: Immunocytochemical characterization of the graft and its integration with the host brain. J Neurosci 7:1626-1638.

Mata M, Fink DJ, Gainer H, Smith CB, Davidsen L, Savaki H, Schwartz WJ, and Sokoloff L (1980) Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J Neurochem 34:213-215.

Millar AJ, Carre IA, Strayer CA, Chua NH, and Kay SA (1995) Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. Science 267:1161-1163.

Moore RY (1973) Retinohypothalamic projection in mammals: A comparative study. Brain Res 49:403-409.

Moore RY and Bernstein ME (1989) Synaptogenesis in the rat suprachiasmatic nucleus demonstrated by electron microscopy and synapsin I immunoreactivity. J Neurosci 9:2151-2162.

Moore RY and Eichler VB (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 42:201-206.

Moore RY, Heller A, Bhatnagar RK, Wurtman RJ, and Axelrod J (1969) Central control of the pineal gland: Visual pathways. Arch Neurol 18:208-218.

Moore RY, Heller A, Wurtman RJ, and Axelrod J (1967) Visual pathway mediating pineal response to environmental light. Science 155:220-223.

Moore RY, Karapas F, and Lenn NJ (1971) A retinohypothalamic projection in the rat. Anat Rec 169:382.

Moore RY and Klein DC (1974) Visual pathways and the central neural control of a circadian rhythm in pineal serotonin *N*-acetyltransferase activity. Brain Res 71:17-33.

Moore RY and Lenn NJ (1972) A retinohypothalamic projection in the rat. J Comp Neurol 146:1-14.

Newman GC and Hospod FE (1986) Rhythm of suprachiasmatic nucleus 2-deoxyglucose uptake in vitro. Brain Res 381:345-350.

Pickard GE, Sollars PJ, Rinchik EM, Nolan PM, and Bucan M (1995) Mutagenesis and behavioral screening for altered circadian activity identifies the mouse mutant, *Wheels*. Brain Res 705:255-266.

Prosser RA and Gillette MU (1989) The mammalian circadian clock in the suprachiasmatic nuclei is reset in vitro by cAMP. J Neurosci 9:1073-1081.

Ralph MR, Foster RG, Davis FC, and Menaker M (1990) Transplanted suprachiasmatic nucleus determines circadian period. Science 247:975-978.

Ralph MR and Menaker M (1985) Bicuculline blocks circadian phase delays but not advances. Brain Res 325:362-365.

Ralph MR and Menaker M (1988) A mutation of the circadian system in golden hamsters. Science 241:1225-1227.

Ralph MR and Mrosovsky N (1992) Behavioral inhibition of circadian responses to light. J Biol Rhythms 7:353-359.

Reppert SM (1995) Interactions between the circadian clocks of mother and fetus. In *CIBA Foundation Symposium: Circadian Clocks and Their Adjustments*, vol 183, DJ Chadwick and K Ackrill, eds, pp 198-211, Wiley, Chichester, UK.

Reppert SM and Schwartz WJ (1983) Maternal coordination of the fetal biological clock in utero. Science 220:969-971.

Reppert SM and Schwartz WJ (1984) Functional activity of the suprachiasmatic nuclei in the fetal primate. Neurosci Lett 46:145-149.

Richter CP (1965) *Biological Clocks in Medicine and Psychiatry*, Charles C Thomas, Springfield, IL.

Rivkees SA and Reppert SM (1990) Entrainment of circadian phase in developing gray short-tailed opossums: Mother vs. environment. Am J Physiol 259:E384-E388.

Rusak B (1977) The role of the suprachiasmatic nuclei in the generation of circadian rhythms in the golden hamster, *Mesocricetus auratus*. J Comp Physiol 118:145-164.

Sawaki Y, Nihonmatsu I, and Kawamura H (1984) Transplantation of the neonatal suprachiasmatic nuclei into rats with complete bilateral suprachiasmatic lesions. Neurosci Res 1:67-72.

Schwartz WJ and Gainer H (1977) Suprachiasmatic nucleus: Use of ^{14}C-labeled deoxyglucose uptake as a functional marker. Science 197:1089-1091.

Schwartz WJ, Sharp FR, Gunn RH, and Evarts EV (1976) Lesions of ascending dopaminergic pathways decrease forebrain glucose uptake. Nature 261:155-157.

Schwartz WJ, Smith CB, Davidsen L, Savaki H, Sokoloff L, Mata M, Fink DJ, and Gainer H (1979). Metabolic mapping of functional activity in the hypothalamo-neurohypophysial system of the rat. Science 205:723-725.

Sehgal A, Price JL, Man B, and Young MW (1994) Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. Science 263:1603-1606.

Shen H, Watanabe M, Tomasiewicz H, Rutishauser U, Magnuson T, and Glass JD (1997) Role of neuronal cell adhesion molecule and polysialic acid in mouse circadian clock function. J Neurosci 17:5221-5229.

Shibata S and Moore RY (1987) Development of neuronal activity in the rat suprachiasmatic nucleus. Brain Res 431:311-315.

Shibata S, Oomura Y, Kita H, and Hattori K (1982) Circadian rhythmic changes in neuronal activity in the suprachiasmatic nucleus of the rat hypothalamic slice. Brain Res 247:154-158.

Silver R, Lehman MN, Gibson M, Gladstone WR, and Bittman EL (1990) Dispersed cell suspensions of fetal SCN restore circadian rhythmicity in SCN-lesioned adult hamsters. Brain Res 525:45-58.

Silver R, LeSauter J, Tresco PA, and Lehman MN (1996) A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature 382:810-813.

Sollars PJ, Ryan A, Ogilvie MD, and Pickard GE (1996) Altered circadian rhythmicity in the *Wocko* mouse, a hyperactive transgenic mutant. Neuroreport 7:1245-1248.

Speh JC and Moore RY (1993) Retinohypothalamic tract development in the hamster and rat. Dev Brain Res 76:171-181.

Stephan FK and Zucker I (1972a) Circadian rhythms in drinking and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci USA 69:1583-1586.

Stephan FK and Zucker I (1972b) Rat drinking rhythms: Central visual pathways and endocrine factors mediating responsiveness to environmental illumination. Physiol Behav 8:315-326.

Stetson MH and Watson-Whitmyre M (1976) Nucleus suprachiasmaticus: The biological clock in the hamster? Science 191:197-199.

Sun ZS, Albrecht U, Zhuchenko O, Bailey J, Eichele G, and Lee CC (1997) RIGUI, a putative mammalian ortholog of the *Drosophila period* gene. Cell 90:1003-1011.

Takahashi JS, Pinto LH, and Vitaterna MH (1994) Forward and reverse genetic approaches to behavior in the mouse. Science 264:1724-1733.

Tei H, Okamura H, Shineyoshi Y, Fukuhara C, Ozawa R, Hirose M, and Sakaki Y (1997) Circadian oscillation of a mammalian homologue of the *Drosophila period* gene. Nature 389:512-516.

Tosini G and Menaker M (1996) Circadian rhythms in cultured mammalian retina. Science 272:419-421.

Udenfriend S (1970) Nobel prize: Three share 1970 award for medical research. Science 170:422-423.

Viswanathan N and Davis FC (1997) Single prenatal injections of melatonin or the D1-dopamine receptor agonist SKF 38393 to pregnant hamsters sets the offsprings' circadian rhythms to phases 180 degrees apart. J Comp Physiol A 180:339-346.

Viswanathan N, Weaver DR, Reppert SM, and Davis FC (1994) Entrainment of the fetal hamster circadian pacemaker by prenatal injections of the dopamine agonist SKF 38393. J Neurosci 14:5393-5398.

Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, and Takahashi JS (1994) Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. Science 264:719-725.

Vitaterna MH and Turek FW (1993) Photoperiodic responses differ among inbred strains of golden hamsters (*Mesocricetus auratus*). Biol Reprod 49:496-501.

Weaver DR and Reppert SM (1987) Maternal-fetal communication of circadian phase in a precocious rodent, the spiny mouse. Am J Physiol 253:E401-E409.

Welsh DK, Logothetis DE, Meister M, and Reppert SM (1995) Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron 14:697-706.

Wurtman RJ, Axelrod J, Chu EW, Heller A, and Moore RY (1967a) Medial forebrain bundle lesions: Blockade of effects of light on rat gonads and pineal. Endocrinology 81:509-514.

Wurtman RJ, Axelrod J, Sedvall G, and Moore RY (1967b) Photic and neural control of the 24-hour norepinephrine rhythm in the rat pineal gland. J Pharmacol Exp Ther 157:487-492.