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13. ABSTRACT (Maximum 200 words)

Over the past three years we have focused our research efforts on the study of the properties of the suprachiasmatic nucleus (SCN) of the tau mutant hamster. In general we have sought to understand how this mutation, which changes the period of circadian rhythmicity from about 24 hours in wild-type animals to near 20 hours in homozygous mutants, affects the SCN itself and how it affects the locomotor behavior which is driven by the SCN. Specifically we have used SCN lesions, which abolish behavioral rhythmicity, followed by transplantation of fetal or neonatal donor SCN, which restores rhythmicity, to ask which components of rhythmic behavior are intrinsic to the SCN and which may depend on its interaction with other structures. We have also studied the free running locomotor rhythms of mutant and wild-type hamsters and compared their responses to constant darkness, constant light and to phase shifting light pulses as a first step toward discovering whether the profound differences that exist in the parameters can all be accounted for by changes in the SCN.

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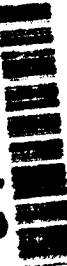
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CONTROL OF CIRCADIAN BEHAVIOR BY TRANSPLANTED SUPRACHIASMATIC NUCLEI

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September 2, 1994

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FINAL TECHNICAL REPORT for period 01 November 1989- 30 April 1994

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SUMMARY

Over the past three years we have focused our research efforts on the study of the properties of the suprachiasmatic nucleus (SCN) of the *tau* mutant hamster. In general we have sought to understand how this mutation, which changes the period of circadian rhythmicity from about 24 hours in wild-type animals to near 20 hours in homozygous mutants, affects the SCN itself and how it affects the locomotor behavior which is driven by the SCN. Specifically we have used SCN lesions, which abolish behavioral rhythmicity, followed by transplantation of fetal or neonatal donor SCN, which restores rhythmicity, to ask which components of rhythmic behavior are intrinsic to the SCN and which may depend on its interaction with other structures. We have also studied the free running locomotor rhythms of mutant and wild-type hamsters and compared their responses to constant darkness, constant light and to phase shifting light pulses as a first step toward discovering whether the profound differences that exist in the parameters can all be accounted for by changes in the SCN.

STATEMENT OF WORK

We will investigate the hypothesis that the mammalian suprachiasmatic nucleus contains the primary driving oscillators in the circadian system by ascertaining whether it is responsible for controlling the period and phase of the overt rhythm of locomotor activity.

We will:

1. Transplant fetal SCN tissue from donors with one of three genetically determined periodicities into hosts that have been rendered arrhythmic by SCN lesion and analyze the periods of the rhythms that are restored by the grafted tissue . We predict that the genotype of the transplanted SCN will alone determine the restored period. This would be strong evidence in favor of the hypothesis.

2. Transplant fetal SCN tissue from donors entrained to different light cycles into arrhythmic SCN-lesioned hosts and analyze the phases of the restored rhythms. We predict that the phase of the restored rhythms will be determined by the phase of the prior entraining light cycle. A positive result from these experiments, coupled with positive results from the period transplant experiments and the extensive information of several different kinds already in the literature would raise the level of confidence in the hypothesis to near certainty.

3. Determine whether multiple SCN oscillators, each with its own genetically determined period, are able to control separate components of the overt circadian behavior, and how they interact with each other. By correlating morphology and behavior in partially lesioned hosts that have received transplants of different genotype than their own, as well as in completely lesioned hosts that have received transplants of more than one genotype, we will investigate what level of interaction among what types of SCN cells leads to coupling among SCN oscillators.

4. Investigate the ontogeny of circadian rhythmicity in the SCN by determining at what age it first expresses Fos-like immunoreactivity in response to light signals and at what embryonic and neonatal ages it can be used successfully to restore circadian rhythmicity to SCN lesioned hosts.

5. Describe the effects of the *tau* mutation on circadian behavior in constant darkness, constant light and in response to phase shifting light pulses. The data generated in this way will be used to build a formal model of the circadian system as modified by the mutation which will hopefully provide insight into the organization of the system in its unmodified forms as well.

RESULTS

1. TRANSPLANTS BETWEEN GENETICALLY DIFFERENT HOSTS AND DONORS

Small neural grafts from the SCN region of the hypothalamus restored circadian rhythm to arrhythmic animals whose own nucleus had been ablated. The restored rhythms always exhibited the period of the donor genotype regardless of the "direction" of the transplant or the genotype of the host. The basic period of the circadian rhythm therefore is determined by cells of the suprachiasmatic region.

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

2. TRANSPLANTATION OF PHASE

We were not able to transplant the phase of the donor's circadian rhythm, however, our results are intriguing. Although not related to the phase of the donor mother's light cycle or to the phase of the surgery itself, the phases of the restored rhythms were not randomly distributed in time. The phases of the rhythms restored by all the donor fetuses in a single litter were clustered (i.e. correlated with each other). One possible interpretation of this result is that at this early stage in development, although the SCN is already rhythmic, its phase is labile and in our experiments was shifted by variable amounts as a result of some poorly controlled aspect of the transplantation procedure. Since we have discovered that successful transplants can be made with tissue from much older animals (as late as postnatal day 12) we are going to try this experiment again using older donors.

3. TRANSPLANTS INTO PARTIALLY LESIONED HOSTS OF DIFFERENT GENOTYPE (TEMPORAL CHIMERAS)

It has been shown previously that a transplant of fetal hypothalamic tissue containing the

suprachiasmatic nuclei to a host rendered arrhythmic by a complete lesion of the suprachiasmatic nuclei restores rhythmicity with the freerunning period that is normally expressed by the donor genotype. We made partial lesions of the suprachiasmatic nuclei of wild-type hosts, which did not completely abolish their circadian rhythmicity, and then placed hypothalamic implants from homozygous mutant fetal donors into the lesion site. The resulting complex patterns of locomotor activity contain rhythmic components with periods of both host and donor circadian oscillators, and suggest the presence of both stimulatory and inhibitory inputs from the circadian system to the centers controlling locomotor behavior.

Vogelbaum MA and Menaker M (1992) Temporal chimeras produced by hypothalamic transplants. *J Neurosci* 12(9):3619-3627

Menaker M and Vogelbaum MA (1993) Mutant circadian period as a marker of suprachiasmatic nucleus function *J Biol Rhythms*, Vol. 8, Supplement, ppS93-S98

Vogelbaum MA, Galef J and Menaker M (1994) Factors determining restoration of circadian behavior by hypothalamic grafts *J Neural Trans and Plas* 4:11-26

4. ONTOGENY OF SCN RHYTHMICITY

Light induction of Fos within the Syrian hamster suprachiasmatic nucleus (SCN) occurred first at postnatal day 4. The number of cells with light-induced Fos-like immunoreactivity (Fos-LI) per unit volume of SCN increased with age. Blinding experiments were used to demonstrate that the eye, though possessing an immature retina, appears to be necessary for light induction of Fos. In neonatal hamsters, environmental cycles (e.g. light and darkness) may be able to reinforce the effect of maternal melatonin in synchronizing the pup's clock.

We have extended our transplant studies in order to evaluate the age range of donor tissue that can be used for transplantation. SCN of hamsters from embryonic day 11 through postnatal day 12 can serve as functional grafts to restore rhythmicity to arrhythmic SCN lesioned animals. The time between SCN transplantation and onset of rhythmicity does not depend on the age of the donor. The presence of patches containing vasoactive intestinal peptide (VIP)

immunoreactive cells is a good indicator of graft success, while its absence is correlated with a lack of transplant effect.. The 18 day span during which SCN tissue can be harvested for transplantation should expand the uses to which this technique can be put. Our results also suggest that it would be advantageous to examine the age range of neural tissue that can be used in other transplantation models.

Kaufman CM and Menaker M (1994) Ontogeny of light-induced Fos-like immunoreactivity in the hamster suprachiasmatic nucleus *Brain Res* 633:162-166

Kaufman CM and Menaker M (1994) Effect of transplanting suprachiasmatic nuclei from donors of different ages into completely SCN-lesioned hamsters *J Neur Trans and Plas* 4(4):1-9

5. EFFECTS OF THE TAU MUTATION ON CIRCADIAN BEHAVIOR

The phenotype by means of which the *tau* mutation in golden hamsters was initially recognized and analyzed genetically was short period length-- ≈ 22 h in heterozygous animals and ≈ 20 h in homozygous ones, in contrast to ≈ 24 h in wild-types. The effect of the mutation in shortening circadian period is localized in the suprachiasmatic nucleus (SCN) and survives transplantation to SCN-lesioned hosts. Based on these observations and experiments we (and others) have conceived of and often referred to this mutation as a "short period" mutation (indeed, informally we refer to heterozygotes as "short" and homozygotes as "supershort" animals). Recent results from our laboratory suggest that this view may be oversimplified and to some degree misleading. It may be more useful to think of the *tau* mutation as destabilizing the mechanism that normally maintains circadian period within narrow limits around 24 hours. Under some--but not all-- environmental conditions, this destabilization results in a shortening of circadian period.

Under some circumstances the circadian period of homozygous *tau* mutant hamsters can become much longer than 20 h. The most dramatic example that has yet occurred is shown in Figure 1. The male hamster that produced this record was held in constant darkness (DD) for

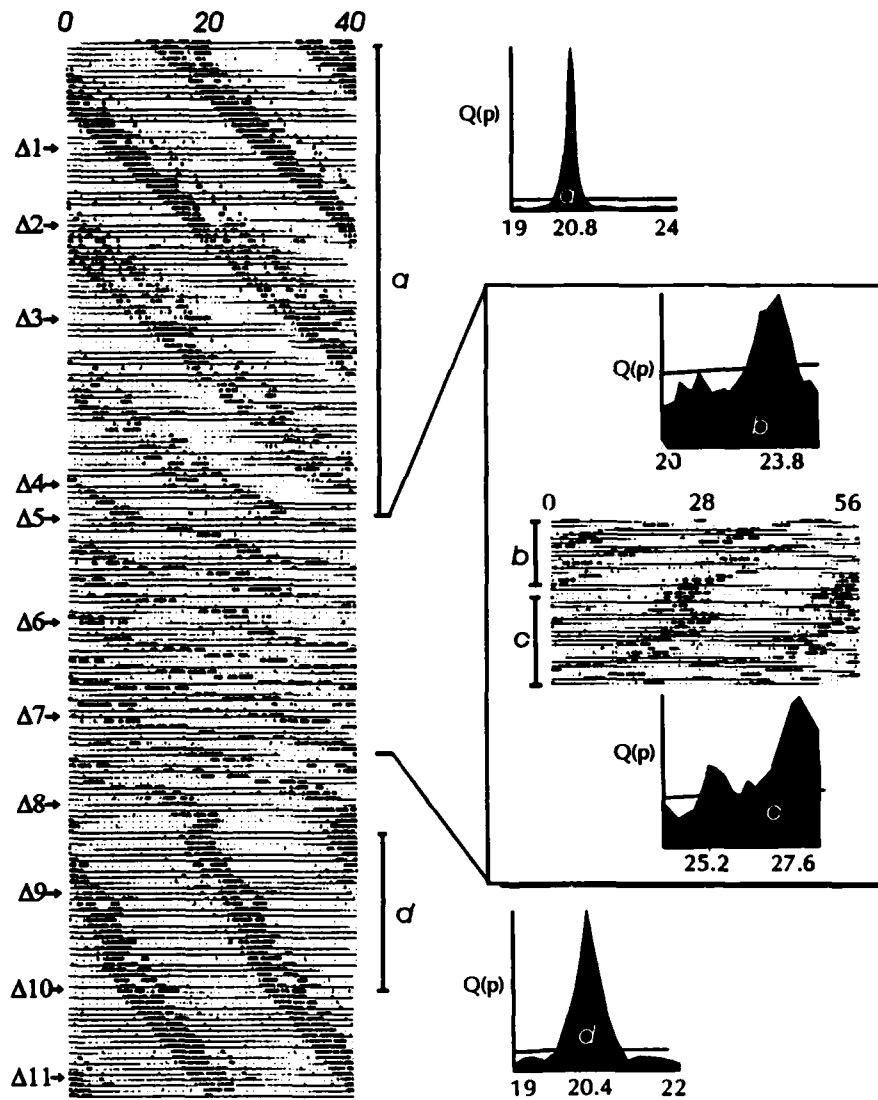


Figure 1 Continuous record of the wheel-running activity of a male homozygous mutant hamster held in constant darkness for about 5 months. In the left-hand panel the entire record has been double plotted on a 20 h time base. On the right-hand side of the figure are shown a segment of data replotted at 28 h and 4 periodograms derived from segments of data identified by the bars a-d. Peaks in the periodograms are considered significant if they rise above the indicated line. Cage changes are indicated by Δ and a number on the far left. Wheel-running activity shows up as dark bars. The unevenness of the background in the activity records is an artifact of the scanning process used to produce the figure and does not indicate missing data.

about 5 months during which time we can assume, based on experiments with other animals and certain features of the record itself (locomotor activity is known to decline in intensity and precision as testosterone declines), that its testes regressed and subsequently recrudesced. In wild-type golden hamsters, complete testis regression in DD (or short daylengths) takes from

10 to 12 weeks; in homozygous *tau* mutants regression takes roughly the same number of *cycles* (thus 20% less absolute time) while the time to the beginning of recrudescence is about 19 - 20 weeks and is unaffected by the mutation. The animal whose record is shown in Figure 1 lengthened its period dramatically beginning after about 87 (20 h) cycles in DD, first to 23.8 h and about 16 cycles later to 27.6 h. It abruptly shortened its period again to 20.4 h (coincident with an increase in intensity and precision) after about 136 cycles in DD. The durations of constant darkness after which these changes occurred correspond so closely to the well documented course of testes regression and recrudescence that the most reasonable interpretation is that they were caused by some aspect of the complex hormonal changes that accompany the changing reproductive state. Note that the cage changes, indicated on the left hand edge of the figure (Δ), while they do not by themselves cause changes in period, are sometimes associated with period changes (Δ 4, 5, 6, 8) and may act to trigger them if other internal conditions are favorable.

Steroid hormones have previously been shown to have small effects on circadian period and activity pattern in rodents. These effects have been interpreted to result from differential effects of testicular hormones on the two oscillators, E and M, which may comprise the circadian pacemaker of rodents or from "slight changes in the phases of multiple oscillators regulating the locomotor rhythm." These interpretations are compatible with each other and with our own view that the *tau* mutation, by destabilizing the circadian system, renders it more susceptible to such influences (see below). Against this background it is not unreasonable to conclude that the very large period changes shown by the homozygous *tau* mutant hamster in Figure 1, are at least in part the result of the changes in testosterone levels that accompany testis regression and recrudescence.

The *tau* mutation also affects the response of the hamsters circadian system to light

pulses. Phase shifts produced by single 1 hour light pulses were compared in homozygous *tau* mutant and wild-type hamsters after several different kinds of pretreatment regimens. There was a dramatic increase in the magnitude of phase delays in mutants as they were kept for progressively longer times in constant darkness (DD) and a smaller increase in the magnitude of phase advances. Under the same conditions a small increase in the magnitude of phase delays and no significant increase in phase advances occurred in wild-type hamsters. After only 7 days in DD the phase response curves (PRC) of mutant and wild-type hamsters were both type 1 and were indistinguishable from each other whereas after 49 days in DD the PRC of mutant hamsters had become type 0.

Mutant hamsters were entrained to eight different T-cycles (one hour of light/cycle), released into DD and given a phase delaying light pulse 7 days later. T-cycles which entrained the animals so that the one hour of light fell between 6 and 9 hours after activity onset suppressed the amplitude of phase delays, while T-cycles which entrained the animals so that the one hour of light fell elsewhere did not suppress phase delays.

Prior environmental (light regimen) history clearly influences the magnitude of light-induced phase shifts in both homozygous mutant and wild-type hamsters. The effects are larger in the delay than in the advance region of the PRC for both genotypes, and much larger in the delay region of the mutant PRC than in this region of the wild-type PRC. What is it that produces these changes? Elsewhere we have argued that the coupling among components of the circadian system may be weakened by the *tau* mutation and that, as a result, phase relationships among oscillators may be altered. Alteration in the phase relationship between E (the oscillator that controls the evening bout of activity) and M (the oscillator that controls the morning bout) has been invoked to explain changes in the activity/rest ratio (α/ρ) and concomitant increases in PRC amplitude. It is therefore of interest to note that the progressive increase in the amplitude

of phase delays produced by light pulses given at CT 14 that occurs with increasing time in DD is closely paralleled by an increase in the duration of α .

In spite of the fact that the changes are manifest when the animals are held for many days in constant darkness, they cannot be attributed to the absence of light *per se*, nor to the fact that the animals are free-running for many cycles. Both of these interpretations are excluded by the results of the T-cycle experiments in which all the experimental animals were exposed to one hour of light per cycle and all those used in the analysis were entrained, yet at the end of the experimental treatment, small phase shifts were shown by animals that had been held on T-cycles of 19, 19.5, 38 and 39 while large phase shifts, indistinguishable from those in DD, were shown by animals that had been held on T-cycles of 20, 20.4, 21.2 and 21.5. The results of the T-cycle experiments also exclude an explanation of the observed changes in response to single light pulses on the basis of age *per se* since all the animals in these experiments were the same age (about five months) when they were tested.

The ability to manipulate the magnitude of the phasic response to single light pulses by controlling prior history of light exposure provides unique experimental opportunities particularly in the *tau* mutant hamster in which such effects are greatly exaggerated. For example it will be of great interest to determine whether changes in the magnitude of behaviorally measured phase shifts are paralleled by changes in the expression of immediate early genes.

Phase response curves to single light pulses were originally sought as support for the analogy between circadian and physical oscillators and were then developed as a way of explaining entrainment to light:dark cycles. The now classic expression $\tau - T = \Delta\phi$ predicts the phase of steady-state entrainment (with reference to "the" PRC) as well as the range of periods to which entrainment is possible with great accuracy in some organisms (e.g., *Drosophila*) but with considerably less accuracy in others such as hamsters. Its efficacy is surely related directly

to the degree to which particular species (and genetic variants within a species!) are subject to the kinds of after-effects that we have observed in exaggerated form in *tau* mutant hamsters. As is particularly clear from our data, one must know in some detail how prior conditions have affected the PRC in order to use it to make quantitative predictions about entrainment. Of course, this is a practical rather than a conceptual limitation on the use of PRCs.

The PRCs of wild-type and *tau* mutant hamsters measured after 7 days in DD are virtually identical when plotted in circadian time. This fact leads to the conclusion that, whatever the mechanism by means of which the *tau* mutation shortens circadian period, its effect is exerted uniformly throughout the circadian cycle. This is perhaps the most important result in the work reported here because it lays heavy constraints on both formal and cellular/molecular models of the *tau* gene's action.

- Menaker M, Shimomura K and Ihara NL (in press) The *tau* mutation destabilizes the circadian system of golden hamsters *Fifth Sapporo Symposium on Biological Rhythms* Hokkaido University Press, Sapporo
Shimomura K and Menaker M (in press) Light-induced phase shifts in *tau* mutant hamsters *J Biol Rhythms*

PUBLICATIONS

- Ralph MR, Foster RG, Davis FC and Menaker M (1990) Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247:975-978
Vogelbaum MA and Menaker M (1992) Temporal chimeras produced by hypothalamic transplants *J Neurosci* 12(9):3619-3627
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Menaker M, Shimomura K and Ihara NL (in press) The *tau* mutation destabilizes the circadian system of golden hamsters *Fifth Sapporo Symposium on Biological Rhythms* Hokkaido University Press, Sapporo
Shimomura K and Menaker M (in press) Light-induced phase shifts in *tau* mutant hamsters *J Biol Rhythms*

PARTICIPATING PROFESSIONALS

Michael Menaker, Ph.D., Principal Investigator

Naomi L. Ihara, M.S., Laboratory specialist

Michael A. Vogelbaum, Graduate student, Ph.D. awarded 1991 "The Temporal Organization of Locomotor Activity in the Golden Hamster: The Case for at Least Two Regulatory Outputs from the Circadian System"

Claire M. Kaufman, Graduate student, Ph.D. awarded 1993 "The Development of the Circadian System in the Golden Hamster"

Kazuhiro Shimomura, D.V.M., Postdoctoral Fellow

INTERACTIONS

INVITED TALKS 1991

University of Virginia Chapter Society of Sigma Xi Seminar, February 26

Foundation for the Study of the Central and Peripheral Nervous System Invited speaker: "The use of mutants in the analysis of mammalian circadian organization," Geneva, Switzerland, April 5

University of Pisa, Dipartimento di Scienze del Comportamento Animale e dell'Uomo, invited lecture: "Circadian Organization in the Vertebrates" April 9

Scuola Normale Superiore, Pisa invited lecture: "The tau Mutation in Hamsters: A Tool for the Exploration of Temporal Order," April 11

International Union of Physiological Sciences Regional Meeting Prague, Czechoslovakia: Symposium lecture "Transplantation of the Suprachiasmatic Nucleus (SCN) Between Tau Mutant and Wild-type Hamsters," July 4

Gordon Conference on Pineal Cell Biology New Hampshire; Discussion Leader: Photic Regulation, August 14

Gordon Conference on Chronobiology; Irsee, Germany, Invited speaker: Circadian photoreception in mammals; Discussant: Endocrine and Neuroendocrine Markers of Human Circadian Clocks, October 03

Max Planck Institut für Verhaltensphysiologie Andechs, Germany, Invited speaker "Reproductive cycles in the tau mutant hamster, October 06

University of Frankfurt Department of Zoology Colloquium: "Temporal Chimeras Produced by Hypothalamic Transplants: The Effects of an Extra Clock," October 08

State University of New York at Stony Brook, Invited speaker: November 04

Society for Neuroscience; New Orleans, LA; Invited speaker in Symposium: How Cells Keep Time: The Molecular and Cellular Basis of Circadian Rhythms. "Transplantation of Mutant SCN Tissue: the Production of Temporal Chimeras" November 13

University of Virginia Science and Cultural Change Symposium invited lecture: 11/20

University of Virginia Department of Biology Seminar: November 22

National Institutes of Health (NICHD) Conference on GnRH Pulse Generation, Invited speaker: "Neuroendocrine Oscillation in the tau-Mutant Hamster," November 25-26

INVITED TALKS 1992

Virginia Commonwealth University Department of Biology Life Sciences Seminar Series: "Circadian Organization in the Vertebrates," February 19

University of North Carolina at Greensboro Department of Biology invited lecture: "Patterns of Circadian Organization in the Vertebrates," April 1

Society for Research on Biological Rhythms Third meeting, Amelia Island, FL, Chair of Workshop: "What do SCN transplant really do?" May 7

University of Virginia College of Arts & Sciences reunion luncheon May 30

Wenner-Gren Center International Symposium on Light and Biological Rhythms in Man, Stockholm, Sweden, invited lecture: "Circadian photoreception in mammals and other vertebrates," Sept. 10

EMBO Workshop on Molecular Chronobiology Leicester, England, invited speaker: "Genetic control of mammalian circadian rhythms: Behavioral effects of transplantation of discrete brain regions," September 27-30

Air Force Office of Scientific Research Chronobiology Review October 2-3, San Antonio, TX, "Control of Circadian Behavior by Transplanted Suprachiasmatic Nuclei"

University of Texas, Austin, Dept. Zoology, First Annual Terrell Hamilton Memorial Lecture, October 5

University of Virginia Biophysics Seminar, "A Mammalian Clock Mutant," November 2

John D and Catherine T MacArthur Foundation Mental Health Research Network on the Psychobiology of Depression and Other Affective Disorders: Task Force Meeting on Biological Rhythms and Psychopathology, Charlottesville, VA, Invited speaker, Nov. 23-24

International Symposium at Yamaguchi University, Japan : Connections Between Genetics and Physiology in the Study of Biological Clocks, invited lecture: "The Physiology of a Clock Mutant Hamster," Nov. 30-Dec.2

NSF Center for Biological Timing Tokyo Symposium, Japan, invited speaker: "Behavioral and physiological analysis of vertebrate circadian rhythms", Dec. 4-6

Nagoya University, Japan, invited speaker, "Uses of the *tau* mutant hamster," Dec. 8

Japanese Neuroscience Meeting, Osaka University, Plenary Lecture: "Circadian Organization in the Vertebrates," Dec. 9

INVITED TALKS 1993

Brigham and Women's Hospital, Endocrine Grand Rounds, invited speaker, "Opportunities for exploration of the timing of endocrine events provided by a circadian mutation," Feb. 10

Clafin College, South Carolina, NSF Center Visiting Lecturer Program, March 23.

Villa-Julie College, Maryland, NSF Center Visiting Lecturer Program, March 30

Neuroscience Graduate Program Seminar Series, UVA, "Temporal Chimeras Produced by Hypothalamic Transplant," April 27.

UVA Health Sciences Center School of Nursing Doctoral Proseminar: "Biological Timing in the ICU" June 16

UVA Mountain Lake Biological Station lecture: "Circadian Organization in the Vertebrates," June 14.

Gordon Conference on Chronobiology, invited commentator, August 9-13.

Fifth Sapporo Symposium on Biological Rhythms: Evolution of the Circadian Clock, Sapporo, Japan, Invited speaker, Symposium 2: Photic and Non-photic Entrainment; Aug. 25-28

Ciba Foundation Symposium No. 183: Circadian clocks and Their Adjustment; London, Sept. 9-11.

University of Virginia Division of Continuing Education Science and Cultural Changes Seminar Series lecture, November 20

NSF Center for Biological Timing: Japan/USA Workshop on Biological Timing, overview lecture "Circadian Systems," December 3

INVITED TALKS 1994

Franklin and Marshall College: Minisymposium of Recent Advances in Circadian Rhythms, "The Tau Mutation in Hamsters: a New Perspective", April 23

Society for Research on Biological Rhythms, fourth meeting, Amelia Island Plantation, Florida, Leader workshop: "Photoreceptors in Vertebrate Circadian Systems", May 7

U.S./Japan Seminar: Cellular and Molecular Basis of Circadian Clocks, Maui, Hawaii, discussion leader: "Exploration of opportunities for international scientific exchange" and chair: "Cellular approaches to the study of circadian timing in non-mammalian models". July 19-21

CONSULTING/ADVISING

Reviewer for Congress of the United States Office of Technology Assessment report *Biological Rhythms and Shift Work*, 1991

NIMH Small Grants Review Panel, February 21-22, 1991

NIMH Intramural Research Program ad hoc consultant for review of Laboratory of Cell Biology, March 7-9, 1991

NIMH Neuroscience Center Review Committee Member, November, 1990-April, 1991

NIMH Intramural Research Program advisor on Neuroethology research program development, September 20, 1991

NIH NICHD Reproductive Sciences Branch, Center for Population Research consultant to Conference on GnRH Pulse Generation, November 25-26; invited speaker "Neuroendocrine Oscillation in the *tau* Mutant Hamster," November 26, 1991

National Institute on Aging Scientific Advisory Committee for *Laboratory for Circadian and Sleep Disorders Medicine* (Harvard Medical School, Dept. Medicine, Div. Endocrinology; C.A. Czeisler, PI) Program Project Grant: "Sleep, Aging and Circadian Rhythm Disorders" 1993

Advisory Committee to *National Institute of Mental Health Workshop on Neuromodulators of Behavior*, Nov 18-19, 1993

NEW DISCOVERIES

Described in published papers

PATENTS

None