REPORT DOCU

AD-A280 977

Form Approved
OMB No 0704-0188

(1)

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		ANNUAL 30 Sep 92 TO 29 Sep 93

4. TITLE AND SUBTITLE

CELL CULTURE AND TRANSPLANTATION OF THE SUPRACHIASMATIC CIRCADIAN PACEMAKER

5. FUNDING NUMBERS

F49620-92-J-0517
61102F
2312
CS

6. AUTHOR(S)

Dr Martin R. Ralph

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Psychology
Univ of Toronto
100 St. George Street
Ontario, CN CANADA

DTIC
ELECTE
JUL 01 1994
S B D

8. PERFORMING ORGANIZATION REPORT NUMBER

AFOSR-TR- 94 0374

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Dr Genevieve M. Haddad
AFOSR/NL
110 DUNCAN AVE SUITE B115
BOLLING AFB DC 20332-0001

10. SPONSORING / MONITORING

94-20249

11. SUPPLEMENTARY NOTES

DTIC QUALITY INSPECTED 2

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

The general aim of the research supported by AFOSR is to understand how circadian rhythms in mammals are generated and controlled. We have used a variety of techniques to ask such questions as: (1) How does photic information reach and affect the clock? (2) What is the nature of the electrical events in pacemaker cells responsible for the generation and expression of rhythmicity? (3) What are the biochemical components of the pacemaker system? In particular, we have used the tau (period) mutation in the golden hamster, to pursue experiments designed to eventually identify mammalian circadian pacemaker cells.

14. SUBJECT TERMS			15. NUMBER OF PAGES
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT (U)	18. SECURITY CLASSIFICATION OF THIS PAGE (U)	19. SECURITY CLASSIFICATION OF ABSTRACT (U)	20. LIMITATION OF ABSTRACT (U)

NSN 7540-01-280-5500

Standard Form 298 (Rev 2-89)
Prescribed by ANSI Std Z39-18
298-102

TECHNICAL REPORT (1992-1993)

The general aim of the research supported by AFOSR is to understand how circadian rhythms in mammals are generated and controlled. We have used a variety of techniques to ask such questions as: (1) How does photic information reach and affect the clock? (2) What is the nature of the electrical events in pacemaker cells responsible for the generation and expression of rhythmicity? (3) What are the biochemical components of the pacemaker system? In particular, we have used the tau (period) mutation in the golden hamster, to pursue experiments designed to eventually identify mammalian circadian pacemaker cells.

A. Transplantation of cultured SCN cells

SCN cells can be maintained in culture for a month or longer. We have found that for up to at least one week, primary cultures are still capable of restoring behavioral circadian rhythms following transplantation. This indicates that pacemaker cells remain viable after short durations in dissociated cell culture and raises the possibility that by eliminating specific cell types from the culture prior to transplantation, the pacemaker cells may be identified. The paper also reports that the signal by which the SCN influences behavior may be inhibitory by nature. These findings are reported in the papers listed below. We have continued to try to dissect the cultures into morphological and antigenic subpopulations. We have had little success in producing restored rhythms when astroglia or neuron enriched cultures are used. These experiments are continuing.

Papers

Ralph, M.R, E.R. Torre and M.N. Lehman (submitted) Culture and transplantation of a mammalian suprachiasmatic circadian pacemaker.

Lehman, M.N. and Ralph, M.R. (1994) Modulation and restitution of circadian rhythms. In: *Functional Neural Transplantation*, (Dunnet, S. and Bjorklund, A., eds), Raven Press, New York. (in press)

Ralph, M.R. and Young, M.W. (1994) The Molecular and Neurophysiologic Basis for Circadian Timekeeping. In: *The Biologic Basis for Optimized Cancer Therapy*, W.J.M. Hrushesky, ed. CRC Press, New York, (in press)

B. Pacemaker-pacemaker communication.

The signals that are produced by transplanted SCN cells may carry a variety of different types of information to the host organism. One signal that has been the subject of numerous research endeavours is the rhythmic output that causes the organism to behave as if it were subjective day or night. The evidence discussed above is that this signal is inhibitory to the behavior of the host. Other signals, that may be quite different in nature, must carry phase

information to the other pacemaker cells in order to produce a coherent rhythm in the overt behavior and physiology.

In young hosts, with partial SCN lesions, chimeric animals may be produce by transplantation, that express the rhythms generated by both donor and host SCN. We and others have shown that these may be expressed simultaneously without any apparent effect of one SCN on the other.

We have found more recently, though, that if the host rhythm has deteriorated (fragmented), an SCN graft may not only be expressed as a new rhythm, but period modulation may be detected as the phase relationship between donor and host rhythms changes. The initial work here was funded by a grant from the NIA. However, it is clear that the effort to characterize the nature of pacemaker communication falls under this grant, and we have incorporated the aged hamster model into our current research. We have found that the presence of the donor SCN may cause large, phase angle dependent phase shifts in the rhythm of the host. This has enabled us to analyze the phase shifting effects of one SCN on another. We now are completing a phase response curve for the pacemaker-pacemaker interaction. It appears that the coupling signal may be non-photic in nature.

Papers

Hurd, M.W., Lehman, M.N. and Ralph, M.R. (submitted) Circadian locomotor rhythms in aged hamsters following suprachiasmatic transplant.

Ralph, M.R., Hurd, M.W., Golombek, D. and Lehman, M.W. (submitted) Pacemaker communication in circadian chimeras produced by SCN transplantation. In: *Advances in Pineal Research*, M. Moller and P. Pevet, eds.

Ralph, M.R. (1994) Circadian pacemakers in vertebrates. In: *Circadian Clocks and Their Adjustment*, K. Ackrill, ed. J. Wiley, Chichester, UK., (in press)

Hurd, M.W. Golombek, D.A., M.N. Lehman, and M.R. Ralph (in preparation) The nature of pacemaker-pacemaker signalling in the mammalian SCN.

C. Role for GABA in the mammalian circadian system

While this experiment was not specifically proposed, we felt that GABA was a reasonable candidate for a pacemaker communication mechanism.

GABA is the most abundant neurotransmitter in the SCN yet the role of this substance in the circadian system has not been well studied. Using pharmacological methods, we were able to construct a simple, testable model for the role of GABA as a modulator of circadian responses to light. Moreover, with few assumptions, we found that GABA cells might couple circadian pacemakers through inhibitory connections. Our more recent work with the inhibition of GABA transaminase confirmed earlier results and suggests that the role of GABA in the SCN might be to regulate the phase-dependent sensitivity of the pacemaker to light.

Papers

Golombek, D.M. and Ralph, M.R. (submitted) Inhibition of GABA transaminase enhances light-induced circadian delays but not advances.

D. Transgenic animals for transplantation studies in circadian rhythms research.

The recent technological advances in applies molecular biology have enabled the production of many lines of mice that carry foreign genes. These have been produced for a variety of reasons and we have been studying the usefulness of many of these in rhythms research. Specifically, one approach has been to use transgenes as markers of grafted cells in SCN transplantation studies. We now have examined the usefulness of eight lines that allow us to identify transplanted cells, and in two cases, to follow neuronal and glial connections with the host.

Three lines carrying v-*jun* driven by the H2K promoter showed nuclear expression within some SCN cells, but the expression was hard to detect. Four lines carrying the *E. coli lacZ* gene appear to be more useful. Two of these show nuclear expression (one in astroglia, the other in neurons and glia). One shows weak cytoplasmic staining mainly in glial cells. And the fourth shows strong cytoplasmic staining in both neurons and glia. Another line carries the entire human neurofilament gene and shows weak staining in SCN neurites. The last two lines were used in some preliminary transplantation studies to determine how easily the donor tissue could be detected in the host background. Both lines appear to be useful.

Abstracts

Ralph, M.R. (1992) Transgenic mice for suprachiasmatic transplant studies. *EMBO Workshop on Molecular Chronobiology*.

Ralph, M.R., Golombek, D.A., Hurd M.W. and Joyner, A.L. (1994) Transgenic markers in SCN transplantation studies in the mouse. *Soc. for Research on Biological Rhythms, Abstr.* 4: 53.

E. Transgenic animals for dissecting the photic entrainment pathway and rhythm generation.

The second approach has been to examine the circadian rhythms and responses to light in animals that carry transgenes or null mutations at genetic loci that are thought to be involved in rhythm regulation. These include many of the immediate-early genes and genes coding for various kinases. Both approaches are actively being pursued.

The rhythms of animals overexpressing *v-jun* are unaffected by the presence of the transgene. However, it is impossible at this time to say whether the foreign product is being expressed in the pacemaker cells.

A line of mice carrying a null mutation at the c-*fos* locus has been studied extensively. Briefly, these animals are still able to respond to light pulses and to entrain to light cycles. Furthermore, their rhythms do not appear to be substantially altered by the gene knockout. The gene may be involved, however, in setting the responsiveness of the system to light. The phase

response curve to light is attenuated in a way that cannot be explained by a reduction in sensitivity to light or damage to the RHT. c-*fos* may act in concert with the other related genes that make up the AP-1 transcription factor. If so, each may have been selected for a subtle effect on the phase relationship with environmental light cycles.

We are now moving toward testing the role of c-*jun* which has a relatively constant background expression in mammals. The knockout is an embryonic lethal. Nonetheless, we hope to be able to examine the adult phenotype by transplanting the embryonic SCN into arrhythmic, SCN lesioned hosts. Since we and others have shown previously that the SCN is essentially the sole determinant of period in rodents, any substantial effect of the gene loss on rhythm generation should be detectable in the recovered rhythms.

Papers

Honrado, G.I., Johnson, R.S., Spiegelman, B.M., Papaioannou, V. and Ralph, M.R. (submitted) The circadian system of c-*fos* deficient mice.

Abstracts

Honrado, G.I., Johnson, R.S., Spiegelman, B.M., Papaioannou, V. and Ralph, M.R. (1994) The circadian system of gene-targeted mice with a null mutation at the c-fos locus. *Soc. for Research on Biological Rhythms, Abstr.* 4: 59.