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During the 1991 contract period we published papers on the effects of clorgyline treatment on photic responses of neurons in the cicadian entrainment pathway, and on the effects of NMDA neurotransmitter antagonists on photic activation of gene expression in the entrainment pathway. We have completed further studies on regulation of messenger RNA levels for several immediate-early genes (IEG) in suprachiasmatic nucleus (SCN) cells in response to light pulses and on the effects of a non-NMDA antagonist on IEG expression in the SCN; the related manuscripts have been accepted for publication. Further studies have been completed and submitted for publication or are currently in preparation. These include studies on: the effects of different temporal patterns of light exposure on SCN cells and on rhythm phase-shifting; antagonism by serotonin of light effects on cells in the circadian system; the effects of bombesin-like peptides on SCN neuronal activity; the effects of several excitatory amino acids on SCN cells in vitro; the regulation of the melatonin sensitivity rhythm of SCN cells; and the roles of nerve growth factor and muscarinic receptor antagonists in modifying circadian responses to cholinergic agents. The results of these studies have either been submitted for publication or are in preparation.

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U.S. AFOSR Contract 90-0104
P.I.: Benjamin Rusak
Annual Technical Report: January, 1992

A. OVERVIEW

The primary goals of the research contract were to investigate the neural mechanisms by which light information is transmitted in the mammalian circadian system, and to study the neurotransmitters involved in photic and other information processing in the suprachiasmatic nuclei (SCN) and intergeniculate leaflet (IGL). The methods proposed to be used in these studies were in vivo and in vitro neurophysiology. Because of the delay in the start of the contract funding relative to our proposed start date, the research associate who was proposed to conduct the majority of these studies had left the laboratory before the contract period began. Replacing him delayed us further because of the initial search and the need for immigration clearance in order to bring the new associate into the country. The new research associate (Guang-Di Yu) arrived in the fall of 1990 and worked here for one year before returning to China; he was replaced immediately with . Shui-Wang Ying, an experienced neurophysiologist, who began work with us in September, 1991.

We requested and received permission from Dr. William Berry, the program manager at the time, to direct our efforts to related topics on which we had obtained exciting new information, pending the arrival of the neurophysiologist. We therefore worked on how gene expression as well as neurophysiological activity are altered in the SCN in response to photic input. Funding was applied to the salary of a postdoctoral associate involved in the gene expression work (Dr. Hiroshi Abe) and to technicians involved in these studies. Although the techniques used in the gene expression studies are different from those in the neurophysiological studies, the overall goals of these lines of work are, in fact, quite similar. Dr. Abe returned to Japan in late 1991, but the neurophysiology laboratory was strengthened with the addition of Dr. Hugh Piggins who joined us early in 1991. Dr. Piggins is a postdoctoral student supported by an NSERC Postdoctoral Fellowship and he has been working on in vitro neurophysiological studies of the SCN.

In addition, we were able to continue our collaborations with Dr. Robert Mason, Dr. Mary Harrington and Dr. Johanna Meijer. During the initial period of support from the AFOSR, we also received a renewal (3 years) of our research grant from the Medical Research Council, which also supports work related to the mammalian entrainment mechanism. Since this support is complementary to that from the AFOSR, and since it has increased by a modest amount, the laboratory should be able to retain the personnel and other resources needed to conduct our research effectively.

B. SPECIFIC TOPICS

1. In vivo Neurophysiology: We completed one collaborative study with Dr. Mary Harrington (Smith College, Northampton, MA) examining the effects of longterm treatment with the antidepressant clorgyline on photic sensitivity curves of cells in the intergeniculate leaflet (IGL). The results indicated that clorgyline pretreatment had no dramatic effects on the shapes of the intensity-response curves, but did lower the overall firing rates of IGL cells both during darkness and in response to light pulses. The manuscript (1) reporting these results was published recently in *Brain Research*.

We have also done a recent study with Dr. Harrington during her visit to

Hamilton in December 1991, exploring the relative photic sensitivities of IGL and SCN cells to light. In this study we used a narrow bandpass filter to allow us to calibrate the light intensity in photons to permit comparison to data on phase-shifting sensitivity of hamsters published by Takahashi, Nelson and colleagues. These data are being analyzed and will be submitted for publication later this year.

We completed a collaborative study with Dr. Johanna Meijer of Leiden University in Holland in which the responses of hamster SCN cells to trains of brief light pulses were compared to their responses to sustained light exposures of equal total duration. These results were in turn compared to the responses of intact animals in terms of phase shifts to similar lighting patterns. The manuscript based on these results has been submitted to *American Journal of Physiology* (2).

In work done by Dexing Zhang and Shui-Wang Ying we have shown that iontophoretic application of serotonin (5HT) or a $5HT_1$ agonist, DPAT, can inhibit the responses to light of IGL cells in vivo. These results are nearly complete and should be submitted for publication in the near future. We are now examining the effects of various antagonists specific for different $5HT_1$ receptor subtypes to identify the type of receptor being affected by 5HT application in the IGL. We are now beginning to address the same issues in the SCN.

2. In vitro Neurophysiology: We have published an abstract with Dr. Mason indicating that prazosin, despite being a potent inhibitor of melatonin binding to one class of melatonin binding sites, fails to block melatonin sensitivity of SCN neurons. These results indicate that a class of melatonin receptor which is not prazosin sensitive is involved in mediating melatonin effects (3).

With Dr. Mason we also published an abstract on the effects of NMDA antagonists on the change in firing rate induced by optic nerve stimulation and on the responses of SCN cells to several types of excitatory amino acid agonists. A manuscript reporting these results has been submitted to *Brain Research* (4).

In studies conducted by Yu Guang-Di during his stay here, we found that the daily rhythm of melatonin sensitivity was abolished by housing hamsters in constant light for 48 hr. We assumed that this result reflected the loss of melatonin secretion, and indicated that the rhythm of melatonin sensitivity was imposed by the presence of circulating melatonin. However, we also noted to our surprise that 48 of constant light also eliminated the daily rhythm of firing rate which we see in SCN cells in vitro. These results make it unclear whether the loss of the melatonin sensitivity rhythm reflects the loss of melatonin or a change in SCN cell function. We therefore repeated the study with pinealectomized animals and again saw a lack of a daily rhythm of melatonin sensitivity after pinealectomy. Pinealectomy did not abolish the daily firing rate rhythm, but the rhythm was attenuated relative to control animals. These results suggest that the melatonin sensitivity rhythm is largely exogenous in origin, but they also raise the intriguing possibility that pineal melatonin plays a role in reinforcing the SCN's spontaneous rhythmicity. We are completing one final control study related to these results and will be submitting two papers to *Brain Research* on this work later this year.

Dr. Piggins has begun to work on the family of bombesin-like peptides which appear to play an important role in SCN function. He is recording from single SCN cells in vitro and applying bombesin and several related members of the bombesin/GRP family and studying their effects on firing rate. Bombesin increases firing in virtually all responsive SCN cells at very low concentrations. He is currently examining which of

the two identified receptor types for bombesin-like peptides is critical to mediating these effects, using both receptor-specific agonists as well as antagonists.

3. NGF and Cholinergics: In another set of studies, K.G. Bina, a graduate student in my laboratory, and Kazue Semba, a colleague in the Dalhousie Anatomy Department have made some important new discoveries in relation to nerve growth factor (NGF) and its receptors in the SCN. Bina is currently writing up her Ph.D. thesis for submission in March, 1992; when this is complete, she will have essentially prepared the manuscripts she plans to submit for publication. These will report on the anatomical origins of the cholinergic and NGF-receptor containing projections to the SCN; the effects of NGF on circadian phase of freerunning activity rhythms; and the blockade of carbachol effects on phase using muscarinic antagonists and anti-NGF antibodies.

4. Gene Expression Studies: Since our initial publication in *Science* of the basic findings that the expression in SCN cells of several immediate-early genes (IEGs), we have continued to pursue related questions, in part with AFOSR support. We (Steven Hunt, Linda McNaughton and Harold Robertson) have shown that the transcription of several immediate-early genes is regulated in a similarly rhythmic fashion. The genes for c-*fos*, c-*jun*, *junB*, *junD*, NGFI-A and NGFI-B are all transcribed in response to a 30 min light pulse during the subjective night in rats, and none of these are transcribed in response to similar light pulses delivered during the subjective day. These results are currently in press in *Molecular Brain Research* (5).

Hiroshi Abe showed that pre-treatment of hamsters with the non-competitive NMDA antagonists MK-801 or ketamine prevented the appearance of Fos protein in cells in most of the SCN. The rostral areas of the SCN, which are normally labeled following light exposure, failed to show any Fos immunoreactivity after MK-801 pretreatment, while Fos labeling in the caudal SCN was reduced in a highly anatomically consistent pattern. The ventral SCN, where labeling density is normally highest, was devoid of label, while the nuclei of cells in a discrete region in the dorsolateral SCN continued to respond to light exposure with strong Fos immunoreactivity. These results suggest that cells in some portions of the SCN depend on an MK-801-sensitive receptor to respond to photic input, while cells in a different region of the SCN can be activated via an MK-801-insensitive route. This finding leads to the novel suggestion that responses of different SCN cell populations to photic input may depend on different transmitters and/or receptor types. These results were presented at the Montreal IBRO meeting and they have been published in *Neuroscience Letters* (6). Abe also showed that more specific competitive antagonists of the NMDA receptor injected into the cerebral ventricles and antagonists that are specific for non-NMDA receptors can also partially block c-*fos* expression in a similar anatomical pattern. These results have been accepted as a Rapid Communciation in *Brain Research Bulletin* and will be published in April of this year (7).

We have also devoted considerable effort to a parametric exploration of the claims we made in our original *Science* paper. Specifically, we have performed immunocytochemical studies for detection of Fos-like immunoreactivity (Fos-lir) and have confirmed that the caudal SCN shows Fos-lir only in response to nocturnal illumination. We have documented the time course of the appearance of the immunoreactivity after a standard light pulse given at a single circadian phase. The intensity of labeling has also been assessed by cell counts and we have found that the

number of cells labeled in response to illumination increases as teh pulse is g en progressively later in the subjective night. We have also shown that when animals are entrained to a non-24 hr photoperiod using a short light pulse, Fos-lir appears in the SCN within an hour after the start of the entraining light pulse.

We have also found that late in the subjective night, robust Fos-lir can be induced by as little as 5 min of light exposure, while during the less-sensitive early subjective night, at least 15-30 min of standard illumination is required to produce a response. We have also assessed whether Fos-lir is induced by dark pulses (when animals are kept in continuous light) given at a phase known to induce large phase advances. We have used a number of different paradigms and approaches and the results have been uniformly negative: Fos-lir is induced by light at night but not by phase-shifting dark pulses during the day. These several parametric studies are currently being prepared for publication.

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