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1) Pineal melatonin may effect the light sensitivity of rats such that pinealectomized rats perceive ambient intensity to be higher than sham-operated controls. We have tested this a several ways. Essentially, we can find no evidence that pinealectomized rats are more sensitive ot light than are pinealectomized rats. We have found that free-running circadian period lengthens in response to increasing light intensities at the same rate, but that pinealectomized rats beocme disrupted at lower intensities than do sham-operated animals. Further, our initial observation that enucleation of rats abolishes SCN iodomelatonin binding ahs proven incorrect when we corrected for circadian phase. 2) Pineal melatonin influences circadian system coupling either at the level of coupling amoung circadian oscillators thenselves orbetween these oscillators ahd there multiple outputs.

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TEXAS A&M UNIVERSITY
Department of Biology

Dr. Genevieve Haddad
Director, AFOSR Chronobiology Program
Bolling Air Force Base
Washington DC 20332-6448

May 12, 1994

Dear Gen,

The past year has been a whirl-wind of progress and success thanks in large part to AFOSR support. I've been waiting to send you this progress report (for AFOSR 91-NL-0244) so that the in press manuscripts listed below had come out, but now I'm sending the proofs and/or manuscripts. I'll send the final papers when I receive off-prints. In all we have published 4 full-length papers (including the AFOSR mini-review which has received more off-print requests than any paper I have ever written-[455 so far!]) and have three manuscripts in press which acknowledge AFOSR support. These are listed below.

1) **Cassone, V.M.**, W.S. Warren, D.S. Brooks and J. Lu (1993) Melatonin, the pineal gland and circadian rhythms. J. Biol. Rhythms 8, Suppl.: S73-S81

2) Warren, W.S., D.B. Hodges, **V.M. Cassone** (1993) Pinealectomized rats entrain and phase-shift to melatonin injection in a dose-dependent manner. J. Biol. Rhythms 8: 233-245

3) Lu, J. and **V.M. Cassone** (1993) Pineal regulation of circadian rhythms of 2-deoxy[^{14}C]glucose uptake and 2[^{125}I]iodomelatonin binding in the visual system of the house sparrow, Passer domesticus. J. Comp. Physiol. A 173: 765-774

4) Lu, J., and **V.M. Cassone** (1993) Daily melatonin administration synchronizes circadian patterns of brain metabolism and behavior in pinealectomized house sparrows, Passer domesticus. J. Comp. Physiol. A 173: 775-782

5) **Cassone, V.M.**, and J. Lu (in press) The pineal gland and avian circadian organization: the neuroendocrine loop. Adv. Pineal Res.

6) Warren, W.S., T.H. Champney and **V.M. Cassone** (in press) The suprachiasmatic nucleus controls circadian rhythms of heart-rate via the sympathetic nervous system. Physiol. Behav.

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7) **V.M. Cassone**, D.S. Brooks, and T.A. Kelm (in press) Comparative distribution of 2[^{125}I]iodomelatonin binding in the avian brain: outgroup analysis with turtles. Brain Behav. Evol.

In addition, we have published one paper which did not acknowledge AFOSR support listed below.

1) Janik, D., **V.M. Cassone**, G.E. Pickard, M. Menaker (1994) Retinohypothalamic projections and immunocytochemical analysis of the suprachiasmatic region of the desert iguana. Dipsosaurus dorsalis. Cell Tiss. Res. 275: 399-406

Furthermore, my students and I have presented 4 abstracts at national meetings since my last progress report. These are listed below.

1) Lu, J., **V.M. Cassone** (1993) Daily melatonin administration synchronizes circadian patterns of brain metabolism and behavior in pinealectomized house sparrows. Neurosci. Abs. 19: 1487

2) Warren, W.S., **V.M. Cassone** (1993) The regulation of multiple circadian outputs by the suprachiasmatic nucleus and sympathetic nervous system. Neurosci. Abs. 19: 1488

3) Warren, W.S., C.L. Cassone, J. Lu, and **V.M. Cassone** (1994) Distribution and daily fluctuations of GFAP immunoreactive astrocytes in the chick visual suprachiasmatic nucleus. Trans. Soc. Res. Biol. Rhythms 4: 118

4) Brooks, D.S., A.J. Mitchell and **V.M. Cassone** (1994) Development of 2-[^{125}I]-iodomelatonin binding in embryonic chick brain. Trans. Soc. Res. Biol. Rhythms 4: 132

Finally, I have been invited to speak at several prestigious meetings and/or University departmental seminar series in the past year. In each case, I have acknowledged AFOSR support. The dates, titles and locations of these talks are listed below.

January 28, 1993 "Melatonin and the Vertebrate Circadian Clock". Department of Neurobiology and Anatomy, University of Texas Health Science Center at Houston, Houston TX

July 23, 1993 "The Pineal Gland and Avian Circadian Organization: The Neuroendocrine Loop". The 6th Colloquium of the European Pineal Society. Panum Institute, Copenhagen, Denmark.

September 6-9, 1993 Discussant, Ciba Foundation Symposium on Circadian Clocks and Their Adjustment. London, England

September 23, 1993 "The Circadian Clock and Cardiovascular Control". Department of Medical Physiology, Texas A&M University

October 14, 1993 "The Role of Melatonin in Seasonal and Circadian Rhythms." Reproductive Science Study Group, Texas A&M University

January 24, 1994 "Avian Circadian Organization" Gordon Research Conference, Casa Serena, CA.

February 22, 1994 "Life, The Universe and Circadian Rhythms". Department of Biology, Texas A&M University

As you can see, we have had an active 1993-1994. Our current projects are even more exciting and have provided new opportunities and directions for the research, although the general research program remains directed at the cellular, molecular, physiological and behavioral effects of the pineal hormone melatonin. I'll list each of these under sub-headings.

Role of the Pineal Gland and Melatonin in Rat Circadian Rhythmicity: Our 1992 J. Biol Rhythms paper (Cassone, 1992, J. Biol. Rhythms 7: 27-40) showing an effect of pinealectomy on the wheel-running activity patterns in rats maintained in constant light (LL) suggested to us two mutually exclusive hypotheses to account for the data:

1) Pineal melatonin may affect the light sensitivity of rats such that pinealectomized rats *perceive* ambient intensity to be higher than sham-operated controls. We have tested this a several ways. Essentially, we can find no evidence that pinealectomized rats are more sensitive to light than are pinealectomized rats. We have found that free-running circadian period lengthens in response to increasing light intensities at the same rate, but that pinealectomized rats become disrupted at lower intensities than do sham-operated animals. Further, our initial observation that enucleation of rats abolishes SCN iodomelatonin binding has proven incorrect when we corrected for circadian phase.

2) Pineal melatonin influences circadian system coupling either at the level of coupling among circadian oscillators themselves or between these oscillators and there multiple outputs. To test this hypothesis, we needed to develop an analytical system to measure multiple circadian rhythms in the same animals. We have done this with AFOSR funds and continue to improve the system with funds recently obtained from Bristol-Myers-Squibb. In order to establish that we can measure multiple rhythms in the same animals, we used the Dataquest III system with heartrate, body temperature and locomotion detecting intraperitoneal probes. In addition, we measured wheel-running activity in our locally designed system. First, we established that SCN lesions abolish all of these rhythms. Second, we demonstrated that the heartrate rhythm can be abolished with sympathetic blockade but not the other rhythms. These data indicate that at least one pathway the SCN clock influences motor output pathways is via the sympathetic nervous system. This paper (Warren et al., 1994, Physiol. Behav. in press) will appear in the next issue of the journal. Now that our system is established, we have addressed whether the pineal gland affects these multiple rhythms. Our hypothesis was as follows: If multiple rhythms become dissociated in LL at the same rate as each other in

pinealectomized rats, the pineal gland must affect intraoscillator coupling. However, if different oscillators uncouple at different rates, the pineal gland affects output pathways. We have just finished this experiment. All four rhythms split or were disrupted in pinealectomized rats at the same rate. These data indicate that the disruption we see in wheel-running activity is likely due to a decrease in coupling strength or rhythm amplitude of the circadian oscillators themselves within the SCN. Together with the lack of effect in gradual intensities of light, we believe we can safely state in published form that, in the rat, the pineal gland alters oscillator properties rather than either photic input or rhythmic output of the clock. We are writing a manuscript (AASERT pre-doctoral fellow Wade Warren is writing it.).

Role of Melatonin in the Avian Circadian System: As you can see from the publication list, we have had some significant success in our work on the avian circadian system, which bear directly on human performance and on the basic mechanisms of circadian oscillation.

1) Effects of Melatonin on the Electroretinogram.

We have found an interesting phenomenon in chicks in which we find a circadian rhythm in the b wave of the electroretinogram (ERG) which is higher during late subjective day than during subjective night. This effect appears to be mediated by melatonin, since exogenous melatonin alters the day-time ERG to a night-time configuration! We are writing a manuscript about this right now, but the data maintains the possibility that melatonin, at least in birds, does indeed alter photic sensitivity, and we are preparing to perform a similar experiment in rats. However, we recognize this will be a much more difficult experiment since the rat eye is significantly smaller than is the chick's (and therefore will generate a much smaller ERG).

2) Daily and Circadian Fluctuations in Astrocytic Processes in the vSCN of Chicks: Effects of Melatonin.

We have confirmed in chicks the incredible phenomenon described by Serviere and colleagues that SCN astrocytes stained for glial fibrillary acidic protein (GFAP) extend their processes during the day and withdraw them at night. We have extended these observations by showing that the chick vSCN glia do this but also that they withdraw their processes in the presence of melatonin. We have one experiment to do. This is to show whether GFAP is rhythmic using Western analysis. Wade Warren and my wife Cyd are doing this right now. Then, they'll write a very cool paper. This study raises the possibility that the actual melatonin receptor may be located on vSCN astroglia. Guess what?

3) The Isolation, Cloning and Anatomical Localization of the Melatonin Receptor in the Chick Brain.

We are collaborating with Steve Reppert and Dave Weaver of Massachusetts General Hospital to clone and express the chick melatonin receptor. Steve and Dave have been able to clone the chick receptor using degenerate primers from Steve's frog receptor. We have done some <u>in situ</u> hybridization and have found preliminarily that the receptor colocalizes with the iodomelatonin binding! I'm flying up to Boston next month to complete this work.

As you can see, there are some very exciting research projects going on in the Cassone laboratory, all of which is due to your generous funding. I hope to write a

renewal proposal by the end of the summer. I believe that it will be crucial for the growth of this program to attract a high quality post-Doctoral fellow to complement my now very productive graduate student training program. I hope you agree. If there are any concerns, questions or friendly comments, please feel free to write, call or, better yet, come and visit. I think you'll be very happy with what you see (Besides, you haven't seen our new house!).

Take care. It was good to see you in Florida, but I didn't get to speak with you enough.

Sincerely Yours,

Vincent M. Cassone, Ph.D.
Associate Professor of Biology