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This research tests the null hypothesis that the restricted phase of melatonin sensitivity in rats is at least partially due to a coincidence of the time at which exogenous melatonin is administered and the time at which endogenous melatonin begins to rise. Results suggest that the cerebral moiety responding to melatonin (presumably the SCN) responds to it quantally within a very restricted phase, and that this effect is independent of the pineal gland. Pinelectomy had no effect on the binding pattern, affinity or capacity within either the SCN or the pars tuberalis.

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Dr. Genevieve Haddad
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March 29, 1992

Dear Dr. Haddad,

This may be astonishing, but this is my research project report for AFOSR grant 90-NL-0244, "Melatonin, the Pineal Gland and Circadian Rhythms" ON TIME. As an aside, I am in somewhat of a quandary as to how I should refer to my renewal. Should I continue with the same number, or should I start afresh? Our Research Foundation has left it in the same account number, so my inclination is to continue with 90-NL-0244. If this is not kosher, please tell me.

Since my last report, 1 paper acknowledging AFOSR support has been published (Cassone, J. Biol. Rhythms 7: 27-40 which will be enclosed in the letter which follows this fax), and 2 papers are in press (1. Cassone et al., in: Advances in Metabolic Mapping Techniques for Brain Imaging of Behavioral and Learning Functions (F. Gonzalez-Lima, H. Scheich and T. Finkenstaedt eds), Kluwer Academic Publishers; 2. Brooks and Cassone, Endocrinology). We are in the process of writing another which will be described below.

The paper currently in preparation describes research performed by my graduate student Wade S. Warren (who is supported by the grant). This research tests the null hypothesis that the restricted phase of melatonin sensitivity in rats is at least partially due to a coincidence of the time at which exogenous melatonin is administered and the time at which endogenous melatonin begins to rise. We have tested this hypothesis by asking whether removal of endogenous melatonin affects the entraining and phase-shifting effects of the hormone. We also felt this was an opportunity to determine whether dose-dependence to exogenous melatonin was affected by the surgery. Rats (N=36 pinealectomized and 36 sham-operated animals) were maintained in constant darkness (DD) and allowed to express their internally driven circadian pattern of wheel-running activity. Wheel-running was recorded with a data acquisition and control computer. Animals received daily injections of 1 of 9 dosages (ranging from 1000 $\mu\text{g}/\text{kg}$ to 0.005 $\mu\text{g}/\text{kg}$) of subcutaneous melatonin every day for 42 days. After this regime, they were allowed to free-run. As stated in my previous August 19, 1991 report, the percentage of rats entraining to the regime were analyzed by probit analysis and indicated no change in the ED_{50} ($4.9 \pm 1.5 \mu\text{g}/\text{kg}$ for shams vs. $5.2 \pm 2.3 \mu\text{g}/\text{kg}$ for pinealectomized). No effect of the surgery was found on the nature of the entrained rhythm nor was there any effect of varying dosages on the detailed aspects of the

activity pattern in this state. This has led us to believe that the cerebral moiety responding to melatonin (presumably the SCN) responds to it quantally within a very restricted phase, and that this effect is independent of the pineal gland. To test this further, we asked whether the amplitude of phase shifts in response to single injections of the hormone were affected by increasing dosages and/or pinealectomy. Free-running rats (N=6/group/dosage) received 1 of 6 dosages ranging from saline control to 1000 $\mu\text{g/kg}$ subcutaneously at Circadian Time 10 (two hrs before activity onset). The data indicated that, like the entrainment effect, pinealectomized rats phase-advanced identically to sham-operated rats and, that, within experimental groups, no graded effect of melatonin was observed. Rats phase-shifted 45 mins or did not, punctuating the idea that the SCN responds to a threshold of melatonin quantally at CT 10. Also, because pinealectomy had no effect on either dose-response relations, it suggested that there was no ligand-receptor down-regulation. To test this idea directly, we have asked whether *in vitro* binding of the melatonin agonist, 2-[^{125}I]iodomelatonin (IMEL), to rat brain sections was affected by pinealectomy. IMEL binding occurs within the SCN, pars tuberalis of the adenohypophysis and several other structures of the rat brain in a high affinity, highly specific manner. In our hands, pinealectomy had no effect on the binding pattern, affinity or capacity within either the SCN or the pars tuberalis.

Wade is currently investigating whether the disruptive effects of pinealectomy I reported this year in constant light (LL) is due to an increase in sensitivity to the 1) parametric effects (changes in period) and/or the non-parametric effects (phase-shifting) of increasing intensities of light. The first experiment is nearly finished. Rats (N=12/surgical group) were maintained in varying intensities of LL ranging from 1×10^{14} to 5×10^{17} photons/cm²/sec. Data analysis is incomplete, and I'd rather not say anything yet.

Two notable collaborations are worth mentioning. These two projects involved the participation of several of my graduate students including Donald Hodges, David Brooks, and Wade Warren and a very bright undergraduate minority scholar Raquel Canales.

In collaboration with Dr. Frank Bronson at the Department of Zoology at University of Texas, we have now conclusively shown that whereas photoperiodic and melatonin sensitive white-footed mice, Peromyscus leucopus, possesses high affinity IMEL binding in the SCN, pars tuberalis and other areas, two species of non-photoperiodic mice, the house mouse (Mus musculus) and the cane mouse (Zygodontomys brevicauda) have either reduced (house mouse) or completely lost (cane mouse) the melatonin receptor. We will be reporting these data to a regional endocrinology conference (the Southwest Regional Conference for Comparative Endocrinology) this weekend.

In collaboration with Dr. Janet Darrow of Northeastern University, we have determined that IMEL binding within the SCN of the Djungarian hamster, Phodopus sungorus, is rhythmic with a peak at CT 10. We are currently asking (experiment is finished, and the data are being analyzed) whether pinealectomy affects this rhythm.

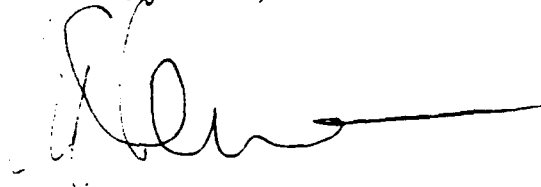
Several projects are imminent. We are currently awaiting the arrival of the infusion pumps and aqueous swivels so that we can continue our *in vivo* microdialysis research. My graduate student Donald B. Hodges has now perfected his amino acid analysis and is ready to measure femtomolar concentrations of aspartate, glutamate, GABA, taurine and other common amino acids from extracellular fluids of SCN and surrounding areas.

We are also awaiting our body temperature and heartrate analysis equipment. Our initial project will test the equipment itself on congenitally blind chickens, since the rat behavioral analysis room will be occupied for at least 4 more months with Wade's light sensitivity studies. This will allow us to work out the bugs in the new equipment and to get some useful data in the bargain.

As you know, I've been invited to speak at this year's Photobiology and Photochemistry meeting (along with Mike Rea and Russ Foster). Also, I've identified the student to whom my Augmentation Award should be given and have offered him the position. This student will inform me of his decision soon.

Well, that's all I have right now. I think things are going very well right now. It is possible that we will have a very productive year in 1992. I hope this adequately fills you in. If you need further information please feel free to call me.

Sincerely Yours,

A handwritten signature in dark ink, appearing to read 'V. Cassone', followed by a long horizontal line extending to the right.

Vincent M. Cassone, PhD
Assistant Professor of Biology

P.S. I've just been promoted with tenure to Associate Professor!