Pineal Melatonin Rhythm: Reduction in Aging Syrian Hamsters

Abstract. *The melatonin content measured by radioimmunoassay of the pineal gland over a 24-hour period (a light:dark cycle of 14 hours of light and 10 of darkness) was compared in young and old female and male Syrian hamsters. The young animals of both sexes exhibited roughly an eightfold rise in pineal melatonin during the dark phase of the cycle, whereas in the old hamsters the nocturnal rise in melatonin was almost completely absent. The results indicate a marked drop in pineal biosynthetic activity in the aging hamster.*

Melatonin (*N*-acetyl-5-methoxytryptamine) is an important constituent in the pineal gland of mammals (*1*). The production of melatonin is cyclic with highest concentrations being present during the dark phase of the light:dark cycle (*2*). The pattern of secretion of melatonin, as reflected by blood concentrations of the indole (*3*) as well as its excretion from the kidney (*4*), follows the same rhythm as does its concentration in the pineal gland.

In the specific case of the Syrian hamster, a marked pineal melatonin rhythm has been defined; as in other species, highest concentrations of melatonin occur during the dark phase of each 24-hour period (*5*). Without exception, studies of pineal melatonin in hamsters have used young, reproductively mature animals. We report that in the aging hamster the pineal melatonin rhythm is greatly altered, suggesting a diminished function of the gland as the animals age.

In our study, concentrations of melatonin in the pineal were compared over 24 hours in young (2-month-old) and old (18-month-old) female and male hamsters (*6*). Animals were kept under light:dark cycles of 14:10 (lights on at 0600 hours). Groups of young females and males were killed at 0800, 1200, 1600, 2000, 2400, and 0400 hours. Since there were fewer old animals, groups were killed at 0800, 2000, 2400, and 0400 hours. All hamsters were killed (*7*) by decapitation, and pineal glands were individually placed into 1-ml portions of chilled 0.1*M* sodium phosphate buffer, *p*H 7.0, containing 0.9 percent sodium chloride. The glands were homogenized by sonification and stored at − 20°C until assayed. Melatonin concentrations were measured by radioimmunoassay of duplicate 200-μl portions from each 1 ml of homogenate (*8*). Mean melatonin concentrations in the pineal were statistically compared by an analysis of variance and a *t*-test.

In both young females and males mean concentrations of pineal melatonin in the daytime were 100 to 125 picograms per gland (Fig. 1). Eight hours after the onset of darkness (0400 hours), the mean values rose to 835 and 887 pg/gland for females and males, respectively. In the old females the daytime concentrations of melatonin were insignificantly lower than those for the young animals. During darkness there was only a very slight rise to 238 pg of melatonin per gland at 0400 hours ($P < .02$ compared to mean values at 0800, 2000, and 2400 hours). During the dark period, the increase in the old females was much less than that in the young animals. Similar observations were made in the old males. After the onset of darkness there was a weak rise in the mean concentration of melatonin at 2400 hours ($P < .05$ compared to mean values at 0800, 2000, and 0400 hours). Thus, the pineal of the old males, like those of the old females, exhibited a depressed melatonin rhythm.

There have been few studies on the pineal gland of aging animals (*9*) and none on this organ in old hamsters. It is well documented that in the Syrian hamster both the pineal gland (*10*) and the melatonin it produces (*11*) can profoundly inhibit the function of the pituitary-gonadal axis. We now present data indicating that the biosynthetic activity of the pineal gland is diminished with age.

Female hamsters deteriorate reproductively between about 11 and 14 months of age while males may sire young until about the age of 20 months (*12*). Since the responses of the pineal were the same in both aging females and males it would seem that the alterations in melatonin production were not related to the reproductive status of the animals. This is consistent with the observations of Rollag *et al.* (*13*) who showed that neither concentrations of circulating pituitary gonadotrophins nor gonadal steroids have a measurable influence on pineal melatonin production in young female hamsters.

The function of most nonreproductive endocrine organs remains essentially unchanged, or they may exhibit a slight depression in advanced age (*14*). Certainly, none is so markedly influenced as is the pineal gland. So little is known about the aging process in hamsters that it is difficult to relate the profound alterations in pineal melatonin synthesis to other functions that may also be exhibiting concurrent changes. Also, whether the phenomenon of depressed melatonin content in the pineal of aging hamsters is characteristic of this species alone remains unexplored.

There are a number of potential explanations for the observed results. Perhaps the catecholamine receptors on the pinealocyte membranes simply lose their responsiveness to norepinephrine, the neurotransmitter involved with stimulating melatonin production (*15*); alternatively, the postganglionic neurons in the pineal may lose their ability to either synthesize or release the neurotransmitter. Other explanations for the depressed melatonin concentrations in the pineal of aging hamsters include a melatonin precursor deficiency (for example, serotonin) or a general inactivity of the enzymes involved in the formation of melatonin (*16*). Whatever the ex-

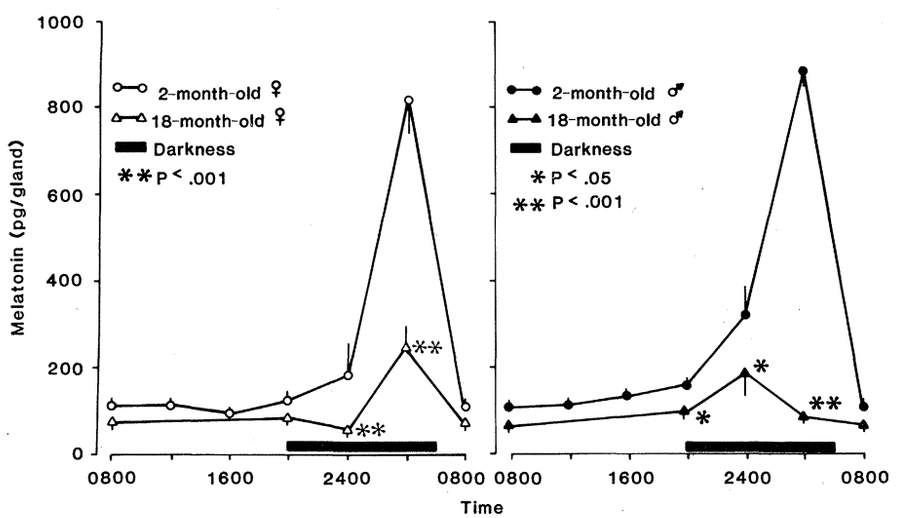

Fig. 1. Pineal melatonin content in young (2-month-old) and old (18-month-old) female and male Syrian hamsters over a 24-hour period. The *P* values indicate differences between young and old at the time points indicated.

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planation, melatonin synthesis and, hence, possibly the function of the pineal, is dramatically reduced in the aging Syrian hamster.

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6. All animals were purchased from Lakeview Hamster Colony, Newfield, N.J. The old animals were maintained in our facility in San Antonio until the young animals were purchased. Thereafter, they were kept under identical environmental and caging conditions. The vaginal cycles of both young and old females were checked by means of post-estrous discharge [M. W. Orsini, *Proc. Anim. Care Panel* **11**, 193 (1961)] for 4 weeks before they were killed. All the young animals exhibited regular 4-day estrous cycles. Somewhat unexpectedly, 20 of 29 old animals also had nearly normal vaginal cycles on the basis of the post-estrous discharge. Some of these had a prolonged cycle; however, most of them had a postovulatory discharge every 4 days. The remaining nine animals were acyclic (at least 10 days without a postestrous discharge).
7. In the case of the young animals, there were eight females and males killed at each time point. For the old males and females, eight each were killed at the two points during darkness (2400 and 0400 hours) while the groups killed during the light phase (0800 and 2000 hours) contained either six or seven females and the same number of males. The total number of hamsters used in the study was 155. Animals killed at night were exposed to a dim red light (25-W tungsten bulb behind a Kodak 1A safe light filter) for 6 to 10 seconds before decapitation. All animals were killed during the same 24-hour period.
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Neural Organization Predicts Stimulus Specificity for a Retained Associative Behavioral Change

Abstract. *Paired, but not random, presentations of light and rotation produced long-term changes in* Hermissenda*'s response to light. The nature of this change depended on the orientation of the animals with respect to the center of rotation and was predicted by known organizational features of* Hermissenda*'s nervous system. When rotation that excited caudal hair cells was paired with light, a significant increase in response latency to test lights resulted. Rotation exciting cephalic hair cells when paired with light decreased the response latencies compared with latencies produced by random presentation of light and rotation.*

Hermissenda will normally move toward a light source in an otherwise unilluminated environment (*1*). This movement can be modified by pairing discrete presentations of light with rotation of the organism (*1*). Paired (but not unpaired or randomly paired) presentations result in significantly longer latencies for the animals to enter illuminated areas when tested immediately and several days after training (*2*). This long-term associative behavioral change is due, at least in part, to primary conductance changes within the somata of type B photoreceptors (*3-5*). The neural organization (*6-8*) of *Hermissenda* (Fig. 1) and stimulus-specific cumulative depolarization of the type B cells (*9, 10*) predict, and this report confirms, that these associative behavioral changes will depend on the intact animal's orientation with respect to the center of rotation.

Hermissenda ($N = 70$) were maintained individually in 15°C seawater (*11*). Each animal was fed daily until satiated on mussel gonad (*Mytilus edulis*) and exposed to 6 ($N = 35$) or 12 ($N = 35$) hours of light daily. Training and testing of animals began after at least 3 days of these maintenance conditions. Training consisted of three phases: (i) baseline assessment of behavior in response to light; (ii) light and rotation regimens; and (iii) multiple reassessments of responses to light. Training and testing techniques have been described (*2, 12, 13*).

The light and rotation schedule began shortly after the end of phase 1 (*14*). Each animal was randomly assigned to one of four treatment conditions. Two groups of animals (paired/caudal and paired/cephalad) received 50 contiguous and completely overlapping pairings of 30 seconds of light and rotation (*15*) on each of three consecutive training days. The time between the initiation of each pairing was, on the average, 2 minutes. Two other groups of animals (random/caudal and random/cephalad) also received 50 30-second presentations of both light and rotation during each of three consecutive daily training sessions. For these animals, light and rotation presentations were presented randomly and independently of one another, although at the same rate (average interstimulus interval for both light and rotation was 2 minutes) as for paired groups. One group of both paired and random animals were secured throughout training with heads oriented toward the center of rotation (paired/caudal and random/caudal) to ensure that the caudal hair cells would be those stimulated by rotation. Similarly, both of the remaining groups (paired/cephalad and random/cephalad) were secured throughout training with heads oriented away from the center of rotation to ensure that the cephalic hair cells would be those stimulated by rotation. After training session 3, each animal was tested for both short- and long-term modifi-

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