nizable pattern of metabolic or functional activity. Thus it is unlikely that the heterogeneity of NADH fluorescence can be attributed solely to variations in metabolic rate.

The alternative explanation for regional heterogeneity of fluorescence is patchy perfusion, which has been suspected in other models of diffuse cerebral oligemia (*6*). The vertically oriented columns of increased NADH separated by a column of normal NADH parallels the orientation of penetrating arteries that descend through the gray matter perpendicular to the surface of the cortex. The pattern of microheterogeneity in cortex suggests either (i) that flow ceases in individual penetrating arteries, while neighboring vessels continue to be perfused or (ii) that there exists between penetrating arteries a microwatershed zone analogous to the macroboundary zone between major cerebral arteries. These findings indicate that the location of the initial alterations in cerebral ischemia is determined largely by vascular factors. However, the location of ultimate cellular damage is likely to depend on additional factors, such as intrinsic vulnerability, which is poorly understood. By using NADH fluorescence as a sampling guide, it should be easier to study the pathological changes in the precise brain regions where ischemic damage is occurring.

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Melatonin Induction of Gonadal Quiescence in Pinealectomized Syrian Hamsters

Abstract. *Pinealectomized Syrian hamsters were injected thrice daily with 25 micrograms of melatonin per injection. The injections were administered at 3-hour intervals either during the day or during the night of a photoperiodic cycle of 14 hours of light and 10 hours of darkness. After 6 weeks of treatment with melatonin during the night, both pinealectomized and intact hamsters had reduced testis weight, and pinealectomized hamsters showed decreased levels of serum gonadotropins. Injection of melatonin during the day for 7 weeks either once (75 micrograms) a day or thrice (25 micrograms per injection) daily caused a reduction in testis weight in pinealectomized hamsters. Both pinealectomized and intact females injected with melatonin thrice daily during the day became anovulatory by week 7 of treatment. These results are similar to those observed when hamsters are exposed to a short photoperiod, suggesting that melatonin may be acting as a hormone in mediating the effects of photoperiod on the reproductive system of the Syrian hamster.*

The pineal gland participates in the regulation of seasonal reproductive cycles in the Syrian hamster (*1*). When hamsters are maintained in photoperiods of less than 12.5 hours of illumination daily, testicular regression in males or a state of anovulation in females ensues after 6 to 8 weeks (*2, 3*). These responses to short photoperiods can be prevented by pinealectomy. Therefore, it has been suggested that in the wild the decreasing day-length of autumn induces the pineal to secrete an "antigonadal" hormone which leads to a state of gonadal quiescence (*4*).

A physiological response similar to that observed in a short photoperiod has been achieved in hamsters kept on a long photoperiod by the implantation of melatonin-filled Silastic capsules (*5*). This observation might be interpreted to suggest that melatonin is the pineal "antigonadal" hormone. However, it should be noted that implantation of melatonin in Silastic capsules or in beeswax pellets prevented gonadal atrophy in hamsters maintained in short photoperiods (*5, 6*). Also, the autoimmunization of hamsters to melatonin induced testicular atrophy (*7*). Thus, the precise role of melatonin is not clear. The animals used in the melatonin implant studies (*5, 6*) were not pinealectomized, so it is possible that (i) exogenous melatonin is additive to or interferes with the action of endogenous melatonin, (ii) exogenous melatonin alters the secretion pattern of endogenous melatonin, or (iii) exogenous melatonin stimulates or inhibits the release of another pineal product, with this second product serving the role of an "antigonadal" hormone.

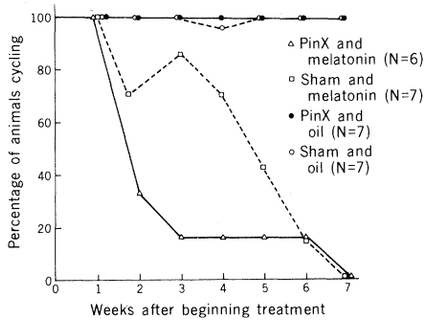

Fig. 1. Effect on estrous cyclicity of daily injections of melatonin (25 μg of melatonin per injection) or 0.05 ml of oil sesame (vehicle) at 1000, 1300, and 1600 hours in pinealectomized (*PinX*) and sham-pinealectomized (*Sham*) female hamsters maintained in a 14L:10D photoperiod. The percentage of females cycling was determined at the end of each week of treatment and injections were administered for 7 weeks.

We have reported that daily injections of melatonin cause gonadal quiescence when administered to hamsters during the afternoon or evening but have no effect when given in the morning (*8*). However, this daily injection regimen failed to inhibit the reproductive systems of pinealectomized hamsters. Thus, for the reasons outlined above it became important to determine whether exogenous melatonin could cause gonadal quiescence in pinealectomized animals. It seemed possible that injections of melatonin given once daily were effective when administered in the afternoon or evening because at those times the exogenous melatonin might be temporally additive to endogenous melatonin (which is produced in greatest quantities during the night in other species). We reasoned that if this were the case, multiple daily injections might be effective in inducing gonadal regression in pinealectomized hamsters.

Hamsters born and reared in our colony were housed in groups and maintained on a photoperiod of 14 hours of light and 10 hours of darkness (14L:10D). Adult animals were pinealectomized by a modification of the technique of Hoffman and Reiter (*9*) and allowed at least 2 weeks of recovery time

before inclusion in an experiment. Daily injections of melatonin (Schwarz/Mann) were administered subcutaneously in 0.05 ml of sesame oil. Females were checked daily for the presence of a vaginal discharge indicative of the day of estrus (*10*). At the end of each treatment, blood samples were obtained from animals of both sexes by cardiac puncture under ether anesthesia. Serum was obtained by centrifugation and stored at −20°C until assayed for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by radioimmunoassay (*11*). After the blood sample was obtained, the left testis of each male in the first experiment and both testes of each male in the second experiment were removed and weighed. A 6-week treatment of thrice-daily injections of 25 μg of melatonin at 2000, 2300, and 0200 hours (with lights on from 0600 to 2000 hours) to pinealectomized male hamsters caused a reduction in testis weight compared to oil-injected, pinealectomized controls (*12*). The thrice-daily regimen of melatonin injections also resulted in testicular regression in sham-pinealectomized males (Table 1).

In a second study, melatonin was again administered three times daily, but during the light period. After 7 weeks of treatment, pinealectomized males injected with 25 μg of melatonin at 1000, 1300, and 1600 hours had markedly reduced testis weights. Pinealectomized males given a single daily injection of 75 μg of melatonin at 1000 hours also had reduced testis weights, but the effect was not as great as when the dosage was divided among three injections. In this experiment the sham-pinealectomized males that received 25 μg of melatonin at 1000, 1300, and 1600 hours did not display significant testicular atrophy (Table 2).

In the second study female hamsters were included, and pinealectomized females injected with melatonin at 1000, 1300, and 1600 hours became anovulatory after 7 weeks of treatment. Sham-pinealectomized females also became acyclic in response to the thrice-daily injection of melatonin (Fig. 1). All the acyclic females from both groups had low concentrations of serum gonadotropins at 1000 hours and increased concentrations at 1500 hours (Table 3). Pinealectomized and sham-pinealectomized females injected thrice daily with sesame oil continued to cycle regularly and only basal levels of serum gonadotropins were observed when these animals were bled at 1000 and 1500 hours on the day after estrus.

Male hamsters induced into a period of testicular regression by exposure to a short photoperiod will undergo testicular recrudescence following removal of the pineal gland. This testicular growth can be prevented by the implantation of melatonin in Silastic capsules at the time of pinealectomy. In the same study, implantation of melatonin resulted in testicular atrophy in pinealectomized hamsters maintained on a long photoperiod (*13*). The present data show that injections of melatonin can also induce gonadal quiescence in pinealectomized hamsters—that is, melatonin need not act by way of stimulation or inhibition of another pineal product. The effect of thrice-daily injections of melatonin on the inhibition of gonadal function in the pinealectomized hamster is accompanied by a reduction in the level of serum gonadotropins in the male and a daily "surge" of serum gonadotropins in the female. Similar patterns in serum gonadotropins have been observed in pineal-intact hamsters exposed to a short photoperiod and also in intact males and females kept on a long photoperiod but receiving single daily injections of melatonin during the afternoon or evening (*3, 8, 14*).

The observations cited here strengthen the hypothesis that melatonin may act as a hormone in mediating the effects of a short photoperiod on the hamster reproductive system. However, some questions remain. First, why did thrice-daily injections of melatonin fail to induce testicular regression in intact hamsters when administered at 1000, 1300, and 1600 hours, even though the same regimen induced regression in pinealectomized males? From our earlier studies we have concluded that both dosage and

Table 1. Left testis weight and concentrations of serum gonadotropins of pinealectomized male hamsters injected once or thrice daily with melatonin during the night.

Dose (μg) per injection	Time of injection (hours)*	*N*	Weight of left testis (mg)	LH (ng/ml)	FSH (ng/ml)
		Pinealectomized hamsters			
Oil	2000, 2300, 0200	5	1835 ± 39†	88 ± 38	167 ± 21
25	2000, 2300, 0200	7	706 ± 225‡	35 ± 3‡	106 ± 7‡
75	2000	7	1420 ± 93	99 ± 20	245 ± 34
		Intact hamsters			
Oil	2000, 2300, 0200	5	1672 ± 63		
25	2000, 2300, 0200	4	543 ± 80‡		

*Lights on from 0600 to 2000 hours. †Mean ± standard error. ‡$P < .01$ compared to oil-injected groups.

Table 2. Combined testes weights and concentrations of serum gonadotropins of pinealectomized male hamsters injected once or thrice daily with melatonin during the day.

Dose (μg) per injection	Time of injection (hours)*	*N*	Combined weights of testes (mg)	LH (ng/ml)	FSH (ng/ml)
		Pinealectomized hamsters			
Oil	1000, 1300, 1600	8	3212 ± 182†	139 ± 34	213 ± 24
25	1000, 1300, 1600	8	633 ± 135‡	110 ± 25	115 ± 32‡
75	1000	7	1198 ± 327‡	49 ± 16‡	86 ± 10‡
		Intact hamsters			
Oil	1000, 1300, 1600	5	2849 ± 127	98 ± 23	181 ± 36
25	1000, 1300, 1600	7	2678 ± 115	95 ± 30	69 ± 9‡

*Lights on from 0600 to 2000 hours. †Mean ± standard error. ‡$P < .01$ compared to oil-injected groups.

Table 3. Concentrations of serum gonadotropins in the morning and in the afternoon in pinealectomized female hamsters injected thrice daily (at 1000, 1300, and 1600 hours) with melatonin during the day. Lights were on from 0600 to 2000 hours.

Dose (μg) per injection	*N*	LH (ng/ml)		FSH (ng/ml)	
		1000 hours	1500 hours	1000 hours	1500 hours
		Pinealectomized hamsters			
Oil	7	27 ± 2*	45 ± 16	227 ± 31	226 ± 32
25	6	29 ± 4	334 ± 12†	134 ± 26	447 ± 24†
		Intact hamsters			
Oil	7	26 ± 1	30 ± 5	259 ± 50	190 ± 28
25	7	< 25	313 ± 28	159 ± 27	603 ± 119†

*Mean ± standard error. †$P < .01$ compared to oil-injected groups.

time-of-day of melatonin treatment is important in determining the effects of this compound in pineal-intact hamsters. Single daily injections of melatonin (75 μg) did induce testicular atrophy when given to pinealectomized males at 1000 hours, whereas the same dosage had little effect when given at 2000 hours. In a previous study with intact males, single daily injections of 50 μg of melatonin administered at 1000 hours had no effect on testicular weight (*15*). These findings suggest that exogenous melatonin may be either potentiated or inhibited by the presence of the pineal (that is, endogenous melatonin?) depending on the time of injection.

Second, why did the same thrice-daily regimen of melatonin which was ineffective in pineal-intact males lead to acyclicity in intact females? In this regard it may be useful to enumerate two observations we have made on the effects of pinealectomy in females: (i) In some of our previous experiments a few females have become anovulatory following pinealectomy alone. (ii) In some experiments females that continued to cycle regularly following pinealectomy became anovulatory after daily injections of sesame oil. Similar problems have never been encountered in our experiments with male hamsters, nor were they encountered in the present study with females. Since maintenance of ovulatory cycles in this species requires regulation by a rhythmic neural center, whereas maintenance of functional testes may not (*16*), it might be that the female reproductive system is in general more subject to interference by systems related to rhythmicity (that is, the pineal) than is the reproductive system of the male. Nevertheless, even in the present experiments thrice-daily injections of melatonin in pinealectomized females appeared to block ovulatory cycles more rapidly then did the same treatment in intact females, providing some degree of analogy to the data for males.

Further studies are required to gain a better understanding of the role of the pineal in hamster reproduction. The finding that gonadal quiescence can be induced in pinealectomized hamsters by treatment with the pineal product, melatonin, should prove useful in further investigations of the role of this compound.

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12. Male hamsters for this experiment were born and reared in an altered 14L:10D photoperiod with lights-on 13 hours earlier than in the colony. For ease of comparison all times are expressed as "colony time" with the lights-on phase at 0600 to 2000 hours. A red fluorescent light was on constantly in this room.
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17. We thank the NIAMDD Rat Pituitary Hormone Program for material used in the FSH assays and for the LH standard. We are also indebted to G. Niswender, and L. Reichert, Jr., for reagent used in the LH assays. Supported by PHS grant HD 05481-06, PHS research training grant MH 14278-02, and the University of Connecticut Research Foundation.
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A Phantom-Motion Aftereffect

Abstract. *Motion aftereffects, typically found to result only from localized retinal stimulation, were obtained within regions of the visual field that had not been stimulated by moving contours. "Phantom" stripes are seen moving through a physically homogeneous (empty) region of the visual field when vertical stripes move above and below that region. Immediately afterward, stationary stripes in the previously empty region appear to move in the opposite direction. This phantom-motion aftereffect provides a novel instance of the way global structure affects processes that have been assumed to be influenced only by simpler local spatial and temporal variables.*

After observers view a pattern of light and dark stripes moving steadily across some region of their field of view, a stationary pattern of similar orientation will appear to move in the opposite direction. This illusion of movement is called the waterfall illusion or the motion aftereffect; it has typically been shown to be restricted to that area of the visual field where the original movement occurred (*1, 2*).

We have found a motion aftereffect that is not restricted to the area of the visual field where the original movement occurred. Our design makes use of Tynan and Sekuler's finding (*3*) that segments of vertical stripes, moving horizontally, above and below a physically homogeneous region, create the impression that vertical stripes or contours are moving through the empty region (moving phantoms). After observing moving phantoms, we found that stationary stripes physically present in the previously empty region appear to move in the opposite direction—a phantom-motion aftereffect.

This phantom-motion aftereffect seems to depend on the presence of perceived motion in the empty region. Moving phantoms are not seen when the moving grating is present only above or only below the empty region, nor when the display is rearranged so that the empty region is vertically rather than horizontally oriented. Under these conditions, we found that reports of a motion aftereffect drop dramatically.

We used two grating patterns to produce the moving phantoms—a square wave grating (Fig. 1A) and an "illusory grating" (Fig. 1B) (*4*). The term "illusory grating" here refers to the stationary dark vertical stripes with apparently continuous contours that are seen as in front of a background of columns of X's (Fig. 1B). Both the real and the illusory gratings produced moving phantoms that in turn produced strong phantom-motion aftereffects (*5, 6*). In addition, when the contrast of the illusory grating is reversed so that black X's move against a white background, a striking amount of detail in the phantoms is observed: one sees not only phantom contours crossing the empty region, but also—dimly, but unmistakably—columns of X's in the empty region. A strong motion aftereffect is obtained in this case also.

Six different moving configurations were used as adapting patterns (Fig. 1, A through F). A square-wave grating (0.36