pulse-echo intervals in orientation by bats makes this example a good illustration of the use of temporal information, and the neurons observed here may eventually lead to detailed knowledge of further neural mechanisms in echolocation. The role of these neurons within the totality of the bat's auditory system is not known from our data.

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Hamster Refractoriness: The Role of Insensitivity of Pineal Target Tissues

Abstract. *Hamsters exposed to short days undergo gonadal collapse followed by recrudescence and insensitivity to the regressive effects of such photoperiods. This refractoriness may be due to exhaustion of the pineal gland or desensitization of its target. Hamsters whose gonads had spontaneously recrudesced were injected with melatonin (25 micrograms per injection) once daily (known to induce regression in intact hamsters) or thrice daily (reported to arrest reproduction in pinealectomized hamsters) for 7 weeks. In neither case did refractory hamsters respond to melatonin treatment. The gonads of intact hamsters treated with melatonin for 21 weeks regressed and spontaneously recrudesced along a normal time course. These results indicate that gonadal refractoriness is due to insensitivity of the target tissues of the pineal gland and imply that melatonin participates in photoperiodic regulation of reproduction in the golden hamster.*

The seasonal breeding cycles of many vertebrates include a period of refractoriness to stimuli that induce gonadal regression at other phases (*1*). Photoperiods of less than 12.5 hours of light per day induce reproductive quiescence in the Syrian golden hamster, *Mesocricetus auratus* (*2*). Either pinealectomy or exposure to long days accelerates recovery of reproductive competence in hamsters whose gonads have regressed, but even the gonads of hamsters maintained on short-day photoperiods recrudesce spontaneously (*3*). Such spontaneous regrowth is thought to account for the return of reproductive activity in nature (*4*).

Hamsters whose testes have recrudesced remain insensitive to the regressive effects of short photoperiods unless they are exposed to at least 10 weeks of long days (*5*). Two alternatives have been offered to account for refractoriness to short days: (i) termination of production of the pineal gland's antigonadal factor (pineal exhaustion hypothesis) or (ii) desensitization of the pineal's target tissues (presumably in the brain) to its hormone (target insensitivity hypothesis) (*4*).

Melatonin, an indole whose rhythmic synthesis is largely restricted to the pineal, has been implicated in photoperiodically induced gonadal regression in both sexes. Daily injections of 10 or 25 μg of melatonin shortly before the end of the light phase induce testicular regression and arrest of the estrous cycle within 7 weeks in intact hamsters maintained in 14-hour photoperiods (LD 14:10) (*6*). Pinealectomized animals are unresponsive to such treatment. Three daily injections of melatonin at 3-hour intervals beginning 4 hours after the onset of light induce testicular regression in pinealectomized but not in intact animals. These differences in sensitivity of intact and pinealectomized hamsters may reflect an interaction between endogenous and exogenous melatonin or a role of the pineal in phasing the responsiveness of target tissues to pineal hormones (*6*).

In the experiments described here I exploited the differences in responsiveness of intact and pinealectomized hamsters to exogenous melatonin in order to determine the etiology of postregression testicular refractoriness. If pineal exhaustion accounts for the insensitivity of the testes to short days, refractory hamsters should be functionally pinealectomized and therefore induced to regress only by thrice daily injections of melatonin. If refractoriness results from insensitivity of target tissues to pineal hormones, such animals should be unresponsive to exogenous melatonin regardless of the timing of its administration. The target insensitivity hypothesis further predicts that the gonads of hamsters induced to regress by melatonin treatments will recrudesce if such injections are continued for several additional weeks. Such an outcome would also imply that spontaneous recrudescence results from the same physiological change responsible for testicular refractoriness. The results of the experiments indicate that the pineal's target tissues do become insensitive to the action of its antigonadal hormone.

Sexually mature male golden hamsters (LAK-LVG) were purchased from the Lakeview hamster colony (Newfield, N.J.) or were bred in our laboratory from similar stock. Animals were housed in groups and maintained under conditions of LD 14:10 (lights on at 0700 hours) prior to experimental use; they were caged singly thereafter (*7*). Testicular condition was monitored by periodic laparotomies (*8*).

Experimental hamsters (N = 44) were laparotomized and divided into two groups matched for body weight and testis index. After an interval of 47 days, one group was transferred to a photoperiod of LD 10:14 (lights on at 1000 hours) while the other was placed in constant darkness (*9*). Laparotomies performed 39 and 210 days later documented regression and spontaneous testicular recru-

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Table 1. Testis indices of hamsters injected with melatonin. Melatonin injections (25 μg/0.1 ml, subcutaneously) began on 17 October in all groups. Results are expressed as means ± standard error. Sample sizes are given in parentheses.

Treatment before injection	Date of laparotomy					
	21 January	16 April	3–17 October	7 December	10–14 February	14 March
			Injected once daily			
LD 10:14	2.28 ± 0.06 (10)	1.05 ± 0.16* (10)	2.17 ± 0.07* (10)	2.11 ± 0.09 (9)		
Constant dark	2.20 ± 0.06 (9)	1.08 ± 0.19* (9)	2.15 ± 0.08* (9)	2.22 ± 0.06 (8)		
Intact, LD 14:10			2.25 ± 0.08 (8)	0.79 ± 0.12*† (7)		2.01 ± 0.08* (6)
Pinx, LD 14:10			2.09 ± 0.09 (10)	2.01 ± 0.07 (9)	1.00 ± 0.19* (7)	
			Injected three times daily			
LD 10:14	2.40 ± 0.10 (10)	1.04 ± 0.14* (10)	2.19 ± 0.09* (10)	2.10 ± 0.06 (8)		
Constant dark	2.38 ± 0.09 (9)	0.84 ± 0.12* (9)	2.14 ± 0.08* (9)	2.17 ± 0.04 (7)		
Intact, LD 14:10			2.24 ± 0.08 (8)	1.44 ± 0.25†‡ (8)	0.54 ± 0.04‡ (6)	
Pinx, LD 14:10			2.07 ± 0.09 (9)	0.59 ± 0.04*† (8)	0.71 ± 0.20 (2)	

*Differed from values obtained in previous laparotomy, dependent t-test, $P < .001$. †Differed from values obtained for all other groups on that date, independent t-test, $P < .005$. ‡Differed from value obtained in previous laparotomy, dependent t-test, $P < .03$.

descence. These animals were then returned to the original LD 14:10 photoperiod and allowed free access to running wheels for 2 weeks. Locomotor activity patterns were recorded in the standard manner (*10*).

Control hamsters of similar age remained in the LD 14:10 photoperiod throughout the experiment. Sixteen of these animals were laparotomized, and 18 were laparotomized and pinealectomized (*11*) at the time the experimental hamsters were placed in running wheels. After a 2-week recovery period, the groups of pinealectomized and intact hamsters were each divided into two subgroups of equivalent mean testis index. Experimental animals were similarly segregated by matching testicular size; two subgroups consisted of hamsters whose testes had regressed and recrudesced in constant darkness while the other two contained those animals that had been maintained in the LD 10:14 photoperiod. At this point all animals were maintained in hanging cages in the same room and subcutaneous injections of 25 μg of melatonin (Sigma) dissolved in 0.1 ml of sesame oil were begun. Of the matched groups of pinealectomized, intact, and recrudesced hamsters, one subgroup received melatonin injections at 1100, 1400, and 1700 hours while the other received daily injections at 2045 hours. After 7 weeks of injections, all hamsters were laparotomized and the testes of representative animals were removed for histological analysis (*12*).

Melatonin injections were continued in those animals that had been maintained on the LD 14:10 photoperiod for the entire experiment (*13*). Treatment was stopped after 16.5 weeks in groups injected three times daily. Of the hamsters injected once per day, the pinealectomized animals were treated for 17 weeks, whereas intact hamsters received a total of 21 weeks of injections. Laparotomies and histological analyses were performed when melatonin treatment ended.

Throughout the experiment testicular indices of hamsters exposed to the LD 10:14 photoperiod were similar to those maintained in constant darkness (Table 1). Reentrainment of activity rhythms was normal and rapid when each of these hamsters was returned to the LD 14:10 room, indicating that all animals received injections at the same intervals after the circadian phase marked by the onset of running. For these reasons, data from these two groups were combined for statistical evaluation.

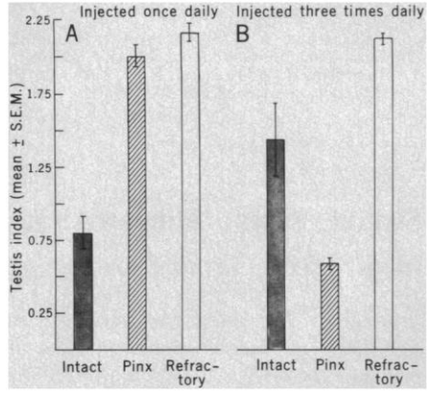

Fig. 1. Testis indices of hamsters injected with melatonin (25 μg per injection) for 7 weeks. Shaded bars represent intact hamsters maintained on photoperiods of LD 14:10 throughout the experiment. Hatched bars represent animals pinealectomized (Pinx) prior to beginning the injections; these animals were also maintained on LD 14:10 photoperiods. Open bars represent hamsters whose testes regressed and spontaneously recrudesced during 30 weeks of exposure to LD 10:14 or constant darkness. These animals were moved to LD 14:10 photoperiods 2 weeks prior to the start of injections. (A) Results of single daily injections: pinealectomized and refractory hamsters' testes were of the same size ($P > .10$), whereas both differed significantly from intact hamsters' testes ($P < .0001$). (B) Results of three daily injections: intact hamsters differ from pinealectomized and refractory animals ($P < .005$ and $P < .002$, respectively). Refractory hamsters' testis indices differed significantly from those of pinealectomized animals ($P < .0001$).

Seven weeks of once-daily melatonin injections timed to occur shortly before the light was turned off caused a dramatic decline of testis indices of intact control hamsters from preinjection values ($P < .001$, Fig. 1). Such treatment did not alter the testicular condition of pinealectomized males. Hamsters whose gonads had regressed and recrudesced prior to melatonin treatment were also uninfluenced by single daily injections.

Three injections per day were more effective in regressing the gonads of pinealectomized than intact hamsters ($P < .005$, Fig. 1). Contrary to the findings of Tamarkin et al. (*6*), some intact hamsters' gonads did regress from preinjection values when the animals were injected three times per day ($P < .02$). Recrudesced testes were significantly less sensitive to this treatment than were the gonads of either the pinealectomized or the intact controls ($P < .002$); there was no evidence of regression in refractory hamsters as a result of 7 weeks of melatonin treatment.

Continuation of melatonin treatments three times daily resulted in more complete gonadal regression in intact control hamsters than had been observed after the first 7 weeks ($P < .03$) or before melatonin treatment began ($P < .001$, Table 1). Pinealectomized hamsters injected once daily for an additional 10 weeks experienced gonadal regression from the large testis indices observed after the initial 7 weeks of injections ($P < .001$, Table 1). Most important is the finding that the testes of intact hamsters, which had regressed in response to 7 weeks of once-daily melatonin injections, uniformly recrudesced when melatonin injections were continued for an additional 14 weeks ($P < .001$). Histological analy-

sis revealed active spermatogenesis when testis indices were large (>1.8) and arrest of spermatogenesis when testis indices fell below 1.0.

The present results agree with and expand upon the findings of Tamarkin *et al.* (*6*), who demonstrated the efficacy of injecting melatonin once and thrice daily in intact and pinealectomized hamsters, respectively. My results indicate that either pattern of injections produces regression in both pinealectomized and intact animals; pinealectomy alters only the time course of the effect. It is not known whether the responsiveness of pinealectomized hamsters to single injections fluctuates with time of day.

In contrast to intact and pinealectomized hamsters, animals whose testes have regressed and spontaneously recrudesced fail to respond within 7 weeks to either regimen of melatonin injections. The difference between these animals and those subjected to pinealectomy refutes the hypothesis that recrudescence and subsequent refractoriness to short days result from pineal exhaustion. The fact that the gonads of hamsters initially induced to regress by daily melatonin treatments spontaneously recover despite continuation of such injections further strengthens the target insensitivity model. There is no need to posit any change in pineal secretory activity during the course of recrudescence. The desensitization of the pineal's target, indicated by these experiments, is adequate to account for both spontaneous recrudescence and the ensuing refractoriness to short days.

There are at least two ways in which such insensitivity might develop. It is possible that the locus at which the pineal hormone acts actually ceases to take up the substance or stops responding to it intracellularly. Alternatively, recovery from suppression of gonadotropin secretion might be a general property of the neuroendocrine system. Both puberty and spontaneous recrudescence may partially reflect spontaneous shifts in steroid feedback threshold from hypersensitive levels (*14*).

Long days might terminate refractoriness by inducing the functional equivalent of pinealectomy. This would be the case if the absence of some pineal principle, perhaps the antigonadotropin itself, were required for the target to regenerate. Alternatively, secretion of melatonin at a phase appropriate to long photoperiods might be involved in restoration of sensitivity to short days. Still another possibility is that the photoperiodic time measurement mechanism might operate directly on the pineal's target to restore its responsiveness.

The present results establish that melatonin loses its ability to suppress reproductive activity during that phase of the breeding cycle characterized by unresponsiveness to short photoperiods. Furthermore, single daily injections of melatonin induce gonadal regression and recrudescence along a time course similar to that elicited by exposure to short days. These findings strengthen the argument that melatonin is a pineal antigonadotropic hormone (*15*) and indicate that target tissue insensitivity, or some other alternative to pineal exhaustion, accounts for refractoriness to short photoperiods.

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Sickling Rates of Human AS Red Cells Infected in vitro with *Plasmodium falciparum* Malaria

Abstract. *The kinetics of sickling of malaria-infected red cells from humans with sickle cell trait were studied in vitro in an attempt to obtain direct experimental evidence for a selective advantage of the hemoglobin S heterozygote in a malarious region. The sickling rates of cells infected with* Plasmodium falciparum *and of noninfected cells were studied both in the total absence of oxygen (by dithionite addition) and at several different concentrations of oxyhemoglobin which might obtain in vivo. In all cases, red cells containing small plasmodium parasite forms (ring forms) sickled approximately eight times as readily as uninfected cells. Cells containing large parasitic forms (trophozoites and schizonts) appeared to sickle less readily than uninfected cells, by light microscopy criteria, but electron micrographs demonstrated the presence of polymerized deoxyhemoglobin S with a high frequency. It is concluded that enhanced sickling of plasmodium-infected AS cells may be one mechanism whereby the hemoglobin S polymorphism is balanced in favor of the heterozygote.*

Epidemiological evidence suggests that hemoglobin S (Hb S) and other red cell polymorphisms protect against the lethal effects of malaria caused by *Plasmodium falciparum*. The overlap of the high gene frequency of Hb S and the geographical location of malaria, as well as the lower parasite counts and decreased mortality in individuals heterozygous for Hb S (*1–3*) (that is, possessing normal hemoglobin, Hb A, as well as Hb S), have been interpreted as proof of this relationship. On the other hand, experimental data to support the notion that Hb S offers protection against malaria are scarce. Perhaps the best experimen-

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