(5). It is also interesting that disinhibition has been tentatively invoked to explain similar changes in the receptive fields of some frog retinal ganglion cells (6). The absence of any response to the tract input at the field center might be due to the restricted area involved. It has been shown that spatial summation is very important in the inhibitory systems of goldfish retinal ganglion cells, to the extent that many complex properties were missed in earlier studies which had employed only small spots for activation (7).

There are other retinal units whose responses to efferent stimulation are a complete contrast with the above, showing enhanced firing to centered spots but remaining quiescent when the whole field is illuminated. In such cases, the centrifugal fibers probably facilitate the excitatory systems which dominate the center of the receptive field.

Closer investigation of these centrifugal effects reveals an increase in the size of the receptive field centers. In order to give prominence to this effect, the surround was activated with an unlimited annulus whose inner margin bordered the excitatory center of the field more closely than was usual in these experiments. In the unit shown in Fig. 3, the annulus came within 0.5° of the center, and gave typical surround responses: flashing the annulus on its own produced no discharge, but when combined with illumination of the center it completely suppressed the normal center responses. As expected, tract stimulation brought out a response to a large-field flash, but in addition, it evoked a response when the annulus alone was flashed. Thus, by suppressing the inhibitory surround, the efferent input uncovers activity from an otherwise silent area, the effect being to increase the apparent extent of the field center. Similar changes have been reported at various levels in the cat visual system (8).

It is difficult to assess the importance of light scatter onto the center in such experiments, and a number of precautions were taken to minimize it (9). If scatter was a major factor in these experiments, then it is likely to have a similar significance in the normal vision of the animal. However, some such expansion of the field center might be expected if the excitatory and inhibitory systems overlap in the boundary regions between the center and the surround, a feature of the Rodieck and Stone proposals. The excitatory field would then extend beyond the center boundaries defined with visual stimuli and would be revealed in its entirety only after an appropriate disturbance in the balance between the excitatory and inhibitory systems. In suppressing the inhibitory system (or facilitating the excitatory system), the centrifugal input could extend the apparent field center.

A number of controls were carried out to discount the possible influence of changes in accommodation and the size of the pupil, eye movements, and circulatory adjustments. In the bird, accommodation can be relaxed and the pupil dilated with drugs similar to curare (10), and the general muscle relaxant used here, a mixture of tubocurare and gallamine triethiodide, was very effective. Microscopic observations of the pupil failed to reveal any changes, and several experiments done with artificial pupils gave essentially the same results. Eye movements were less than 20 minutes of arc per hour, and although occasional drifts followed tract stimulation, they were always small (rarely more than 10 minutes of arc) and of longer latency (several hundred milliseconds) than the effects here described. It was therefore unlikely that eye movement affected the results in any essential way. Nonetheless, considerable care was used in the selection and positioning of spots and annuli so that such small movements could be tolerated. Arterial blood pressure was monitored continuously throughout the experiments, and although it was sensitive to tract stimulation on occasions, the results showed no associated effects.

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Pineal Function and Oviposition in Japanese Quail: Superior Cervical Ganglionectomy and Photoperiod

Abstract. *Bilateral ablation of the superior cervical ganglia appears to deprive the pineal body of sympathetic innervation. Although this procedure presumably interrupts the neural circuit for transmission of optic information to the pineal, oviposition rates of ganglionectomized females exposed to stimulatory (15-hour) or to nonstimulatory (4-hour) daily photoperiods do not differ from those of the controls.*

Pineal organs extend from or occur within the dorsal roof of the brain, specifically the diencephalon, of almost all vertebrates examined. There is great diversity in the origin and organization of these structures, indicative perhaps of diverse functions. The function of the pineal body of birds is obscure, although there is some evidence from gallinaceous species that it may be a gland producing a progonadal agent in very young birds and an antigonadal agent in older birds (1). Interpretations of function of the avian pineal are strongly influenced by assumptions regarding pineal function in mammals, where it is generally held that the pineal acts to suppress gonadal function (2), although proof of this contention remains equivocal (3).

The effect of light (or the absence of light) on the gonads of mammals is believed to be mediated to some extent by the pineal; light information is re-

Table 1. Frequency of oviposition (mean number of eggs per bird per week) of *Coturnix* by subgroups. All operations were performed at the end of experimental week 1, and the birds were killed at the end of experimental week 19.

Age of birds (weeks)	Experimental week	Group 1 UC*	Group 1 SC†	Group 1 GX‡	Group 2 UC§	Group 2 SC‖	Group 2 GX¶
		12-hour light			*12-hour light*		
6	1	4.5	4.9	5.4	3.7	4.5	4.8
7	2	5.7	5.3	4.9	5.9	5.6	4.9
8	3	6.2	5.3	3.5	5.6	3.1	2.8
		15-hour light			*4-hour light*		
9	4	5.3	6.0	5.9	6.0	5.7	5.6
10	5	6.7	6.2	5.7	4.2	4.4	3.8
11	6	5.8	6.8	6.2	2.2	2.5	3.6
12	7	5.6	5.9	5.3	2.3	2.1	1.0
13	8	5.9	6.2	5.3	1.9	1.4	1.1
14	9	5.8	6.4	5.7	3.0	2.1	1.4
15	10	5.6	5.6	5.8	1.8	1.4	1.1
16	11	5.8	5.6	5.7	1.9	2.5	1.7
17	12	5.8	6.0	5.8	1.5	2.5	1.8
		4-hour light			*15-hour light*		
18	13	5.9	5.7	5.0	1.7	2.1	1.5
19	14	4.7	5.7	4.8	2.9	2.7	2.5
20	15	4.8	5.3	3.9	3.6	2.7	3.9
21	16	5.0	4.3	3.9	4.6	4.2	4.4
22	17	5.0	5.6	3.8	5.6	6.0	5.4
23	18	4.3	4.9	5.0	6.0	5.4	6.0
24	19	4.4	4.4	4.6	5.3	5.4	5.5

* $N = 12$ from week 1 to 12; $N = 11$ from week 13 to 19. † $N = 11$ from week 1 to 12; $N = 10$ from week 13 to 19. ‡ $N = 13$ from week 1 to 12; $N = 11$ from week 13 to 19. § $N = 12$ from week 1 to 12; $N = 11$ from week 13 to 19. ‖ $N = 12$ from week 1 to 12; $N = 11$ from week 13 to 19. ¶ $N = 13$ from week 1 to 12; $N = 11$ from week 13 to 19.

layed from the eyes by way of a circuit that involves the superior cervical ganglia and enters the pineal body through postganglionic fibers (*2*). Melatonin, a methoxyindole unique to the pineal in mammals, is the foremost candidate for chemical mediator of this pineal influence (*4*).

If the pineal of homeothermal vertebrates is related to reproductive processes, and particularly to photic influences thereon, birds should be excellent subjects for study, since the effects of light on the reproductive state are so dramatically expressed in most birds (*5*). Domesticated birds are well established as subjects for studies on reproduction, especially females in which frequency of oviposition is a convenient indicator of the reproductive state. We therefore have investigated the possibility that the pineal is a regulator of reproductivity in birds, using female Japanese quail (*Coturnix coturnix japonica*) as experimental subjects. The effect of sympathetic denervation of the pineal by superior cervical ganglionectomy on melatonin content of the pineal body and on responsiveness of the reproductive system to stimulatory and nonstimulatory light periods was examined.

Six-week-old female quail ($N = 73$) of a strain previously described (*6*) were placed singly in sections of four-deck laying batteries and were given free access to game bird chow (Purina) and water. Cool white fluorescent ceiling lamps (intensity 600 erg cm^{-2} sec^{-1} at the center of the room and at the middle level of the battery) were on from 6 a.m. to 6 p.m. When they were 7 weeks old about one-third of the birds were bilaterally superior cervical ganglionectomized (GX animals) (*7*). Success of the operation was evidenced by bilateral ptosis of eyelids. Another one-third were submitted to the same operation, but the ganglia were not removed [sham-operated control (SC animals)]. The remaining one-third served as unoperated controls (UC animals).

Two weeks after surgery half of the birds were subjected to 15 hours of light (from 4 a.m. to 7 p.m.) per day (group 1) and the other half to 4 hours of light (1 p.m. to 5 p.m.) per day (group 2). Both groups contained three subgroups—about equal numbers of GX, SC, and UC birds. After 9 weeks the light regimens were switched. Group 1 received 4 hours of light per day and group 2 received 15 hours of light per day. At this time one or two birds from each subgroup were killed, and their pineals were examined by fluorescence histochemistry. Egg lay was recorded daily for each bird throughout the experiment. Seven weeks after the switch in lighting regimens, all birds were killed by decapitation. Pineal bodies of approximately half of the *Coturnix* in each subgroup were analyzed for melatonin content by a melanophore (frog larva) bioassay (*8*), and those of the other half were prepared for visualization of monoamines by the formaldehyde-induced (histochemical) technique of Falck and Owman (*9, 10*). An attempt was made to include in both studies birds that were laying at high, medium, and low rates. Weights of ovaries and lengths of oviducts of birds used for histology were determined at the time of sacrifice.

There was no significant difference in the mean number of eggs laid per week per bird (Table 1). (*Coturnix* subjected to 14 to 18 hours of light per day begin to ovulate and oviposit almost daily at 6 to 7 weeks of age and continue to do so for several months thereafter.) Birds in group 1, initially on long light periods for 9 weeks, maintained a high average rate of oviposition throughout; when switched to short light periods all three subgroups diminished their rate of oviposition only slightly. Birds in group 2, when placed in short light periods, showed the expected reduction in egg production in all subgroups; when switched to long light periods for 7 weeks, all three subgroups increased their rate of lay to normal in about 5 weeks. Ovarian weight and oviducal length were directly related to oviposition rate and showed no mean difference among subgroups within group 1 or 2.

Bilateral ganglionectomy, when done as unilateral, two-stage operations, has been reported to cause egg production by laying Japanese quail to decline, on the average, to somewhat less than one-half the normal rate for about 2 weeks following removal of the second ganglion, after which they regained normal production (*11*). We obtained similar results; ganglionectomized birds produced fewer eggs than controls during the 2 weeks after surgery (Table 1). Unlike the former authors, who regard this effect as significant, we are of the opinion that it is probably not a specific response to denervation of the pineal body for the following reasons. (i) Sham-operated controls also showed diminished production (Table 1), although not as much as ganglionectomized birds (except SC of group 2 in week 2 after surgery when frequency of lay was about as low as in GX animals). (ii) Surgical trauma results in temporary reduction or cessation of ovulation in chickens (*12*), and removal of the superior cervical ganglia, which provide extensive innervation to the head region, might be expected to be more traumatic than any sham opera-

Table 2. Semiquantitative estimate of fluorescence of representative histological sections of each of the pineal bodies prepared for visualization of catecholamines at the end of experimental week 19. Symbols are: +++++, high; ++++, medium-high; +++, medium; ++, medium-low; and +, absent.

Group 1	Group 2
Unoperated controls	
+++++	+++++
+++++	+++++
+++++	+++++
+++++	++++
++++	++++
++	
Sham-operated controls	
+++++	+++++
+++++	+++++
++++	+++++
++++	+++++
++++	++++
+++	
Superior cervical ganglionectomized	
+++	++
++	+
++	+
+	+
+	+
+	+

Table 3. Melatonin content in pineals of Japanese quail after 7 weeks of short (group 1, 4-hour) or long (group 2, 15-hour) light periods. The birds were killed between 10 and 11 p.m. Not significant, N.S.

Melatonin content (ng/pineal)	
Group 1	Group 2
Unoperated control	
0.0	1.6
0.5	3.2
1.6	5.0
2.0	4.2
2.0	5.0
3.2	
Mean 1.5 ± 0.5	3.5 ± 0.6
Sham-operated control	
0.5	2.0
0.6	3.2
0.6	3.2
2 4	3.2
2.8	3.6
	4.2
Mean 1.4 ± 0.5	3.2 ± 0.3
Ganglionectomized birds	
0.0	0.3
0.2	0.3
0.5	2.0
1.8	2.8
1.8	2.8
2.4	4.2
Mean 1.1 ± 0.4	2.1 ± 0.6

Source of variation	d.f.	Sum of squares	Significance of F (*P*)
Operation	2	3.90	N.S.
Light period	1	21.62	<0.005
Interaction	2	1.95	N.S.
Error	30	53.06	

tion in which nerves are not severed. (iii) The transient and graded (lay not stopped, only reduced) nature of the effect makes it difficult to suggest that permanent sympathetic denervation of the pineal is the cause of it.

The degree of sympathetic denervation was assessed by microscopic examination of sections of pineals prepared by the Falck-Owman technique (*9*). Hedlund (*13*) has shown that in *Coturnix*, as well as in two other gallinaceous species, the adrenergic innervation of the pineal body is derived primarily, and probably exclusively, from the superior cervical ganglia. An arbitrary scale representing the amount of green fluorescence in several typical sections was used to represent semiquantitatively the degree of sympathetic innervation of the pineals (Table 2). In all but one bird (group 1, GX), ganglionectomized quail have pineals that are almost devoid of green fluorescence (catecholamines) and therefore are presumed to be largely or completely without sympathetic innervation. However, there is no correlation between rate of oviposition (Table 1) and apparent extent of sympathetic innervation (Table 2); thus, ganglionectomized *Coturnix*, although deprived of most or all pineal sympathetics, responded to changes in photoperiod like *Coturnix* with normally innervated pineals.

Birds for which pineal melatonin content was determined were killed in the dark between 10 p.m. and 11 p.m., a time during which melatonin content is predictably high (*6*). *Coturnix* subjected to 4 hours of light per day for the last 7 weeks had less pineal melatonin than did birds in comparable subgroups exposed to 15 hours of light (Table 3). However, the effects of the operation on melatonin content were not significant, and there is no significant interaction of lighting and operation (Table 3). It was previously demonstrated (*14*) that ganglionectomized quail appear to retain a pineal melatonin rhythm in that, like normal animals, the content of melatonin was higher in pineals removed during the dark period than in pineals removed during light; however, the concentrations at both times were lower than normal.

These results, taken together, suggest that (i) when excised after 6 weeks of age, the superior cervical ganglia are not essential for normal oviposition in Japanese quail; (ii) although bilateral superior cervical ganglionectomy effectively reduces or abolishes sympathetic innervation of the pineal body and presumably severs the pathway for optic input to the pineal, the normal influence of light on reproductive state and phasing of the melatonin rhythm persists; and (iii) reduction in catecholamine content of the pineal, as effected by ganglionectomy, does not abolish the rhythm and has no noticeable effect on ovarian function, despite the fact that norepinephrine stimulates melatonin formation by pineals in tissue culture and presumably influences activity of one or more of the enzymes involved in melatonin formation (*15*).

It appears that ovarian function, pineal sympathetic innervation, and pineal melatonin show a high degree of independence in female Japanese quail. Expected differences between intact and denervated pineals in photic responsiveness of the reproductive system were not revealed.

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