ferior colliculus. Although the change was again small it was greater than the change produced by white noise.

It should not have been surprising that the nature of the sound stimulus used is critical in electrophysiological studies of the auditory system. Starr and Livingston (*4*) have pointed out the profound differences in distribution of activity evoked by clicks or other transients, as compared with the distribution of activity evoked by sustained white noise. The data reported here emphasize the differences between "white" noise and tonal stimuli, and the distinctiveness of the response patterns of the auditory nuclei.

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Light: Evidence for Its Direct Effect on Hypothalamic Neurons

Abstract. *Continuous light impinging on the suprachiasmatic region of enucleate rats resulted in a constant estrous-like vaginal cycle. The lowest precentage of cornified cells was obtained from exposure of the arcuate or mammillary neurons to light. Ovarian and pituitary weight increased significantly after exposure of the arcuate region to continuous light.*

Environmental lighting profoundly affects pituitary-gonadal function in many vertebrates. A number of workers have shown that the estrous cycle of the female rat is extremely sensitive to the lighting regime (*1*). The problem is thus a search for the receptors.

Benoit and co-workers (*2*) in a series of investigations on the duck have indicated that, even after optic enucleation, light focused on the pituitary, hypothalamus, or rhinencephalon was effective in stimulating the gonads. Recently Ganong and co-workers (*3*) have shown that sunlight can penetrate the hypothalamus in the brains of sheep, dog, rabbit, and rat. Our experiments are an attempt to determine whether light can alter the estrous rhythm in the rat, if the light impinges directly on the hypothalamus.

We observed the following effects, presumably due to light being transmitted directly, by way of glass rods (optic fibers), onto hypothalamic neurons. When light was directed to the suprachiasmatic region of enucleate rats, there was a significant increase in the number of days during which cornified cells were found in the vaginal washings. The data obtained from the vaginal washings are graphically presented in Fig. 1.

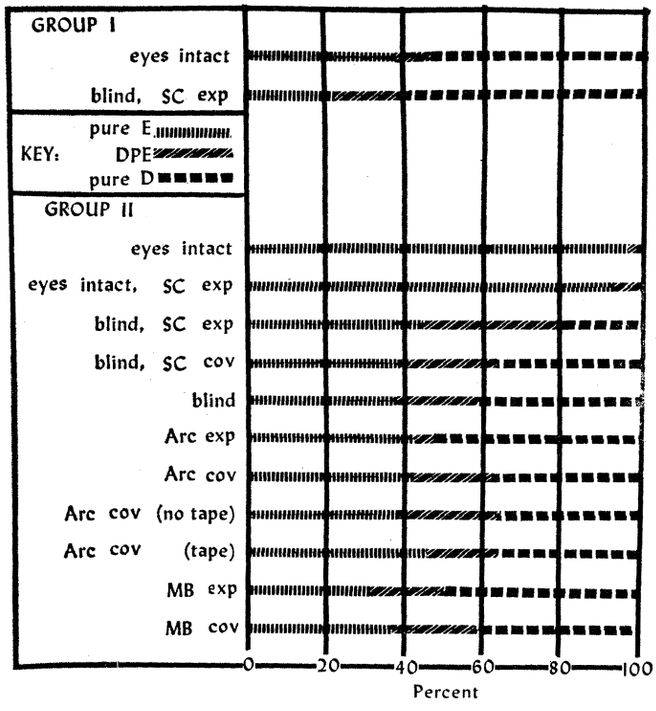
These data were analyzed by the chi-square test and are based on the cell types present in the vaginal smear during the last 20 days of the 6-week experimental period. The analysis showed that there was no change in the number of days on which cornified material was present when cycles of light were presented to the animal by way of the eyes or optic fibers in the suprachiasmatic region. Under constant light a similar relation held for these two groups. However, under constant light, cornified cells were present in over 80 percent of the smears, compared with 40 percent under cyclic light. The only other animals showing a significant change from the animals that were only blind or in experimental control groups were those to which light was transmitted by way of optic fibers onto either the arcuate neurons or the mammillary nuclei. In both instances the chi-square test indicated a highly significant decrease in the number of days with cornified material; cornified cells being found on 45 percent of the days in both these experimental groups, compared to 60 percent in the animals that were only blind or those with fiber implants in other regions of the hypothalamus.

Ovarian weight did not change as a result of constant light on the suprachiasmatic region. However, application of this light by way of the eyes resulted in an average decrease of 42 percent in ovarian weight. Ovaries from the animals with eyes intact contained mostly large follicles, whereas after light had been administered by way of the optic fibers some corpora lutea were also found.

The ovarian weight increased 33 percent when light was directed to the arcuate region. The controls showed no change, nor did the ovarian weight change for any other experimental

Table 1. Effect of light, directed to the hypothalamic neurons, on the weight (wet) of pituitaries and ovaries of rats. The significance of the results are shown by *t*-tests. Figures after weights represent the standard errors of the mean. S, Suprachiasmatic; A, arcuate; M, mammillary; exp, exposed; cov, covered.

Treatment		Animals (No.)	Weight		*t*-test	
Blinded	Placement of rod		Pituitary (mg)	Ovary (mg)	Pituitary	Ovary
		Group I. Cyclic light (14:10)				
−	None	8	13.4 ± 0.88	81.7 ± 3.95		
+	None	5	13.6 ± 0.41	73.2 ± 5.05		
+	S	5	13.3 ± 0.40	98.4 ± 9.18		
		Group II. Constant light				
−	None	6	13.7 ± 0.55	46.6 ± 2.47		
−	S	9	14.4 ± 0.26	46.9 ± 2.49	.001	.001
+	S exp	9	12.2 ± 0.39	79.5 ± 2.01		
+	S cov	8	12.3 ± 0.44	88.4 ± 3.23	−	−
+	None	6	12.1 ± 0.22	77.6 ± 2.48	−	−
+	A exp	5	14.3 ± 0.81	106.6 ± 6.49	.01	.001
+	A cov	8	12.6 ± 0.56	79.5 ± 2.37	.01	
+	A cov + tape	5	11.6 ± 0.59	81.1 ± 2.32		
+	M exp	7	11.7 ± 0.30	87.7 ± 3.46	.01	
+	M cov	6	13.0 ± 0.48	90.8 ± 2.93		



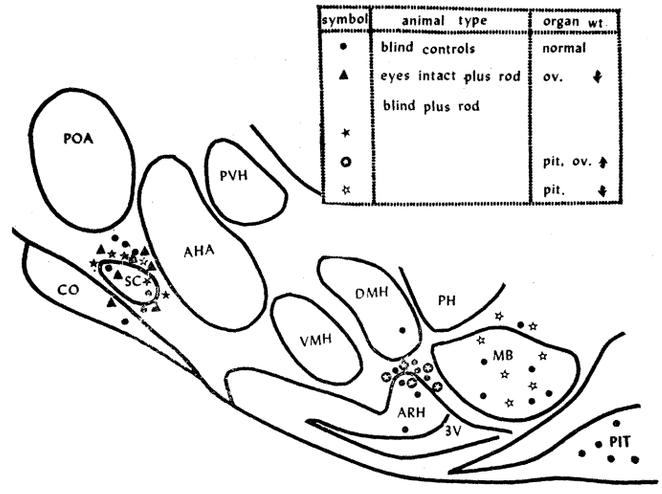


Fig. 1 (left). Frequency distribution of cell types in the vaginal washings obtained daily on the last 20 days of a 6-week experimental period. *E*, Cornified cells; *P*, nucleated epithelium; *D*, leucocytes; *SC*, suprachiasmatic; *Arc*, arcuate; *MB*, mammillary bodies; *exp*, optic fiber exposed to light; *cov*, fiber covered by dental cement. Fig. 2 (above). Location of optic fiber implants in rat hypothalamus with a summary of resultant weight changes in ovary and pituitary. Abbreviations as in Fig. 1.

group. Ovaries from the arcuate-exposed animals were almost entirely corpora lutea; only one large follicle per ovary was found. Microscopic examination of the ovarian interstitial tissue showed "cartwheel" nuclei, an indication of decreased output of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary (*4*).

When three different light intensities were applied to the arcuate region there was a graded increase in pituitary weight, the greatest increase resulting from the most intense light. These pituitaries tended to have increased numbers of gonadotroph cells which were in one instance hypertrophied, with a few castration cells being present. The changes found in ovarian and pituitary weight are summarized in Fig. 2, and the statistical analyses are shown in Table 1.

Both the increased ovarian and pituitary weights after exposure of the arcuate region to constant light, coupled with a histological picture of increased gonadotrophs in the pituitary, and an ovary dominated by corpora lutea and altered interstitial tissue, indicate a change in the cyclic release of FSH and LH. This picture suggests a domination of the pituitary-ovarian axis by luteotrophic hormone (LTH).

As far as we are aware, these are the first experiments which show that light acting directly on hypothalamic neurons can exert a physiological effect in a mammal. In all experiments the rats used were the CFE strain (*5*). Experiments were started, when the animals were 9 weeks of age, by the animals being exposed to constant light for 6 weeks. Vaginal washings were examined daily. The criterion for constant estrus was a positive estrous smear for 9 of 10 consecutive days. Approximately 90 percent of the animals met this standard. The animals were then returned to periodic illumination until the normal estrous cycle was reinstated. At this stage the animals were 15 weeks old.

Optic fibers (outside diameter, 400 μ) were subsequently implanted by the stereotaxic method in various regions of the hypothalamus. The fiber projected about 1 cm above the skull surface and was protected by a mound of dental cement (*5*). For control experiments the rod was clipped off so that it was level with the skull, a layer of black masking tape was applied, and a further layer of dental cement was added. Constant illumination was provided by double fluorescent tubes placed about 20 cm above the animal's head. The lamps used (*5*) had a continuous spectrum with the major peak of energy in the yellow-orange region. All experiments were of 6 weeks' duration.

These experiments appear to indicate that light falling on components of the central nervous system in a mammal may affect physiological function elsewhere. Although the extreme sensitivity of the eye as a light receptor in the functioning of the estrous cycle cannot be negated, it appears that the central nervous system of the mammal can still respond directly to visible radiation. Furthermore, it is worthy of note that light appears to act on those very same regions of the central nervous system in which the sex steroids have been shown to influence the estrous cycle and behavior in the rat (*6*). Thus the neurons responsive to environmental lighting and the hormonal status of the animal would appear to be positioned for maximum interactions, thus leading to the fine control necessary in reproductive function.

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