Table 2. Mean preference for novel food: choice between grape juice and milk. The following effects were significant (F test): novel food ($p < .01$), irradiation ($p < .001$), and novel food by irradiation interaction ($p < .02$). All other effects had $p > .25$. The irradiation effect was significant when milk was novel ($p < .001$) but was marginal when grape juice was novel ($p < .08$). (In the context of the other results, this last probability, being two-tailed, may be considered evidence of reliability.) Preference expressed as in Table 1.

Conditioning procedure	Preferences	
	Irradiates	Controls
Novel food: grape juice		
f→n	0.056	0.119
n→f	.010	.109
Novel food: milk		
f→n	.052	.350
n→f	.026	.267

in a second experiment in which undiluted grape juice (Welch brand) was substituted for the sucrose solution used in the first experiment. In this second experiment, preference for the novel food was reliably lower among the irradiated rats regardless of which food was novel (Table 2). When saccharin and water are presented to the rats before they are irradiated, their aversion to these fluids is attenuated if these fluids are already familiar to them (*8*). Thus the greater associative strength of novel foods in our experiment appears to be a general principle of conditioning.

The rats exhibited neophobia in both experiments. During the conditioning trial, the rats tended to drink the novel fluid more slowly than the familiar fluid (Table 3) except when sucrose was novel; this exception may be attributed to the greater palatability of sucrose solution (Table 1). Furthermore, during the test day of each experiment, the preference for milk among the controls was reliably greater if it was familiar than if it was novel. In view of this prominent role of neophobia, the radiation effect reported here may have been obtained not because of any selective association of the novel food with radiation effects but because a history of illness increases neophobia. This possibility may be discounted because a novel food presented more than a day after x-irradiation will not be avoided (*9*). Thus the apparent role of neophobia is to enhance the association of unusual gastrointestinal events with previously ingested novel foods. How it does so is unknown, but one possibility may be discounted. The increased time with which the animal is usually in contact with the animal food probably is not responsible for the selective association of illness with it; when sucrose was novel, the rats showed the radiation effect even though most of them drank the sucrose as quickly as the milk. Furthermore, among the individual irradiated rats in this category, there was no correlation between the time they took to drink sucrose divided by the time they took to drink milk and the later preference which they showed for sucrose ($p > .25$, Spearman r).

Table 3. Proportion of rats requiring more time to drink the novel fluid than the familiar fluid on the day of conditioning (both experiments). Except when sucrose was novel, each proportion shown was reliably greater than the chance level of .500 ($p < .001$, sign test).

Novel food	Familiar food	Proportion
Sucrose	Milk	0.417
Milk	Sucrose	.958
Grape juice	Milk	.958
Milk	Grape juice	.917

The rats were not fed or given water from the time they ate the test foods until 6 hours later. Therefore, if any aftertastes were present while the illness began, the aftertaste of the food last ingested should have been stronger. If such aftertastes help rats associate what they have eaten with later illness, the aversion for the novel food should be greater under f→n procedure than under n→f procedure. The fact that our experiments did not show this result (Tables 1 and 2), together with other findings (*2*), seems to indicate that aftertastes are not primarily responsible for the ability of rats to associate ingestion with later illness.

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Visual Pathway Mediating Pineal Response to Environmental Light

Abstract. *Activity of the melatonin-forming enzyme, hydroxyindole-O-methyltransferase, in rat pineal is increased when the animal is exposed to continuous darkness, and it is decreased by exposure to continuous light. Response to environmental light is initiated in the retina and transmitted to the pineal by way of the central nervous system and the cervical sympathetics. The central visual pathway essential for mediation of this response is the inferior accessory optic tract. Visual pathways to thalamus and tectum do not participate in this response.*

The mammalian pineal gland contains a unique enzyme, hydroxyindole-*O*-methyltransferase (HIOMT), which transfers a methyl group of *S*-adenosylmethionine to the hydroxy group of *N*-acetylserotonin to form melatonin (*1*). Activity of this enzyme in rat pineal is controlled by environmental lighting. Synthesis of melatonin is depressed in animals kept in continuous illumination but increased in animals maintained in darkness (*2*). Information about environmental lighting is transmitted to the pineal from the retina by way of the brain and the sympathetic nervous system (*3*). In rats blinded by removal of both eyes the response to light by pineal HIOMT is lost, as is the response after denervation of the pineal by superior cervical ganglionectomy (*3*). Similarly, central lesions which bilaterally transect the medial forebrain bundle in the lateral hypothalamus produce a loss of the pineal HIOMT response (*4*). This suggests that a critical component of the central retinal projection is present either within or closely adjacent to the medial forebrain bundle. Anatomy of the retinal projection has been studied extensively in the rat (*5*). Axons of

retinal ganglion cells enter the optic nerve and separate at the optic chiasm into several components. The largest of these is the primary optic tract, which supplies afferent visual input to the lateral geniculate nuclei, the pretectal nuclei, and the superior colliculi. The primary optic tract contains both crossed and uncrossed fibers from the retina. In addition to the primary optic tract there are two accessory optic tracts, the superior and inferior fasciculi of the accessory optic system, which undergo a complete decussation in the chiasm. The superior fasciculus accompanies the primary optic tract to the level of the superior colliculus before diverging to innervate three terminal nuclei within the midbrain tegmentum. The inferior fasciculus, on the other hand, separates from the primary tract just beyond the optic chiasm and enters the lateral hypothalamus to run caudally among the fibers of the medial forebrain bundle before terminating in the rostral midbrain tegmentum (*5*). In several studies retinohypothalamic fibers that leave the chiasm dorsally to terminate in the adjacent medial hypothalamus have also been described (*6*), but other studies have failed to confirm this finding (*5*), which places the existence of this retinal projection in doubt.

We designed this experiment to determine the components of the retinal projection that control the pineal response to light. Utilizing the light-induced changes in HIOMT activity (*3*), we demonstrated that the integrity of the inferior accessory optic tract is essential for the transmission of photic information to the pineal gland regardless of whether other components of the central retinal projections are intact.

Operations were performed on 66 female albino rats of the Holtzman strain (80 to 85 days old) under ether anesthesia; 22 unoperated animals were used as normal controls. Thirty days later half of the rats of each group were placed in continuous darkness or continuous light for 30 days (see *7*). Animals were killed by neck fracture and the pineal glands were removed and individually assayed for HIOMT activity (*8*). Brains from the lesioned animals were removed, fixed in 10 percent formaldehyde, and prepared for histologic study (*9*). Four surgical procedures were performed on the following groups of animals; components of the visual system involved in each procedure are shown diagrammatically in Fig. 1.

1) Animals were subjected to bilateral orbital enucleation.

2) A unilateral lesion was placed in the medial forebrain bundle behind the optic chiasm (*9*) to transect the inferior accessory optic tract originating in the opposite eye. This is accomplished because the inferior accessory optic tract runs within the medial forebrain bundle after its decussation in the optic chiasm. The contralateral inferior accessory optic tract was destroyed at its origin by enucleating the eye on the same side as the medial forebrain bundle lesion. Thus, the inferior accessory optic tract was ablated bilaterally; one primary optic tract (originating in the eye which was not enucleated, Fig. 1) and one medial forebrain bundle were preserved. The side on which the lesions were made was varied systematically from right to left.

3) Large, bilateral lesions were placed in the caudal, lateral thalamus at the level of the lateral geniculate nuclei. Stereotaxic coordinates for this lesion were posterior 3.5 mm from the bregma and lateral 3 and 4 mm from the midline. At each lateral placement a lesion was made 5 and 6 mm below the skull so that there were a total of four lesions on each side. Lesions were produced by passing an anodal d-c current of 2 ma for 45 seconds through an insulated nichrome wire electrode that was bare at the tip. Lesions in these animals were designed to remove all light input to the terminal nuclei of the primary optic tracts (that is, the lateral geniculate nuclei, the pretectal nuclei, and the superior colliculi), as well as to the dorsal and lateral terminal nuclei of the accessory optic system. Animals in this group were also subjected to a unilateral orbital enucleation; hence the only visual pathways remaining intact were one inferior accessory optic tract and any

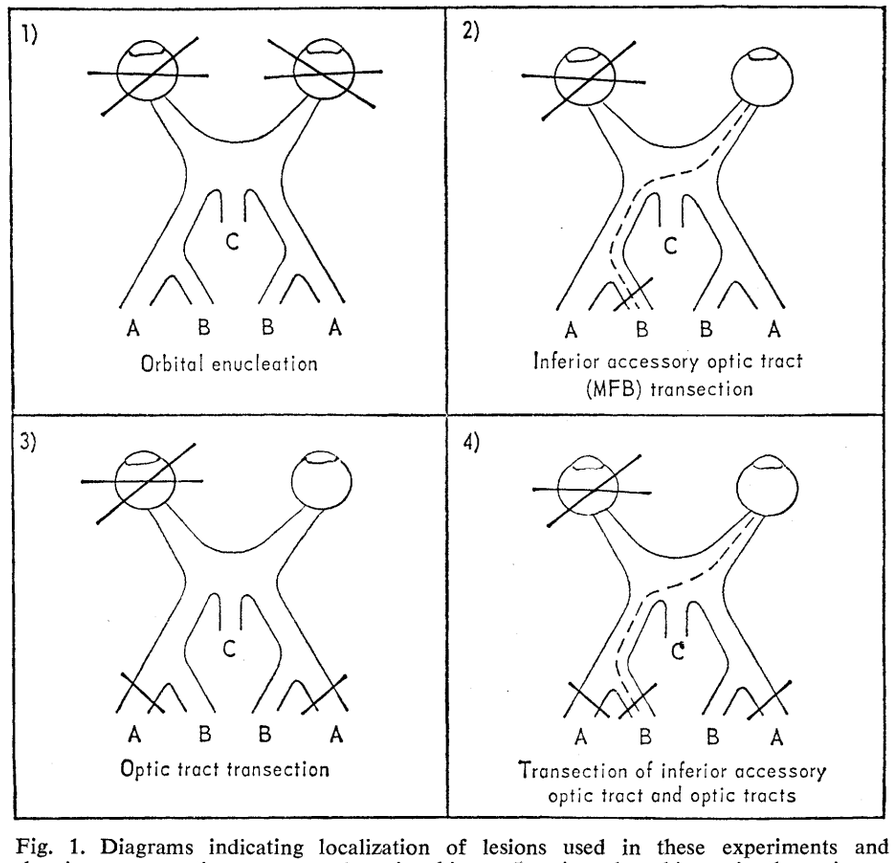

Fig. 1. Diagrams indicating localization of lesions used in these experiments and showing eyes, optic nerves, and optic chiasm. Leaving the chiasm is the primary optic tract (*A*), the inferior accessory optic tract (*B*) which runs in the medial forebrain bundle (*MFB*), and the proposed retinohypothalamic fibers (*C*). In group 1 both eyes were enucleated, removing all central retinal projections. In group 2 the inferior accessory optic tracts were bilaterally removed; the left eye was enucleated, destroying the right inferior accessory optic tract. The left tract (dotted line) was removed by a lesion in the medial forebrain bundle at *B*. The primary optic tract on the left, however, was intact. In group 3 one eye was enucleated, and both primary optic tracts were ablated. Group 4 combines the lesions of groups 2 and 3.

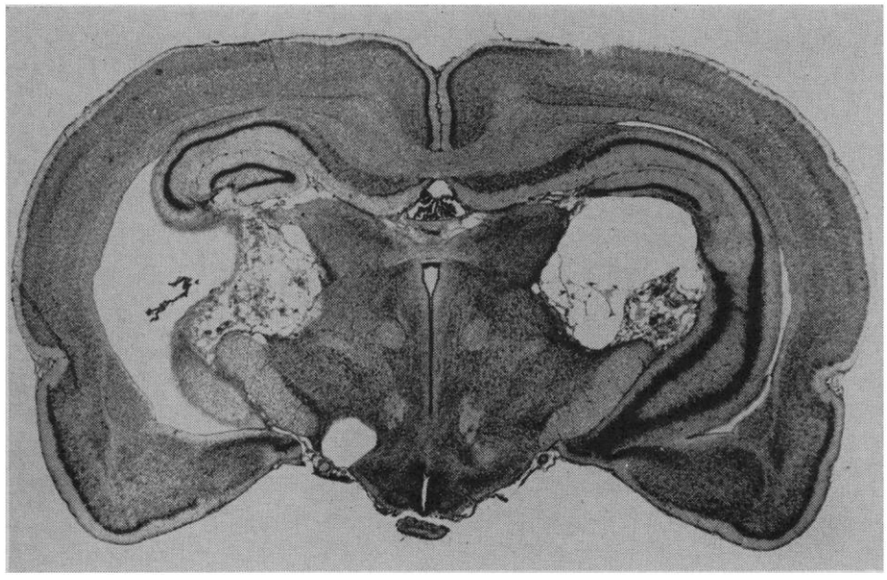

Fig. 2. Photograph of a representative lesion from group 4. Large thalamic lesions are present bilaterally ablating the lateral geniculate nuclei and adjacent structures. In addition there is a unilateral lesion transecting the left medial forebrain bundle. Cresyl violet stain; × 10.

retinohypothalamic fibers that might be present (*5*).

4) Lesions of groups 2 and 3 were combined in this group. This combination would ablate the primary optic tracts and both accessory optic tracts. Since the visual projection to the optic chiasm remained intact, the pineal HIOMT response should have been preserved if retinohypothalamic fibers exist and are important for its maintenance. This group of animals also served as a control for the possibility that either the primary optic tract or the inferior accessory optic tract might be able to maintain the pineal response in the absence of the other.

Data from analyses of the pineal HIOMT are presented in Table 1. Unoperated control rats showed a characteristic change in pineal HIOMT when exposed to constant light or darkness. The melatonin-forming enzyme activity of rats kept in darkness was more than twice that of animals kept in light. Blinding suppressed the effects of light on this enzyme, which confirms previous findings (*3*). Bilateral destruction of the inferior accessory optic tracts also abolished the effects of illumination on pineal HIOMT. These effects persisted, however, in animals in whom the primary optic tracts and the superior accessory optic tracts had been destroyed (group 3). Presence of an intact visual pathway as far as the optic chiasm (group 4) was insufficient to maintain the effect of light on pineal HIOMT. This indicates that if retinohypothalamic fibers exist, they do not mediate this effect of light on the pineal.

At the time of operation, animals were selected for the optic tract group (group 3) if the pupil in their remaining eye was dilated and unresponsive to light. These animals behaved like the blinded group in failing to perceive obstacles and precipices visually. Despite their apparent lack of a behavioral response to visual stimuli, animals in this group showed a normal pineal HIOMT response to light. In contrast to this, animals with section of the inferior accessory optic tract showed a normal response to visual stimuli but no pineal response to alterations in environmental light. Animals with combined lesions, like the blinded group, demonstrated a lack of both behavioral responses to visual stimuli and a pineal response.

Table 1. Effect of visual pathway lesions on the response of pineal hydroxyindole-*O*-methyltransferase (HIOMT) to continuous light or darkness. Groups of 9 to 11 rats were subjected to various surgical procedures. Thirty days later they were placed in continuous light or darkness for 30 days and their pineals were assayed for HIOMT. Values given are ± the standard error.

Condition	HIOMT activity ($\mu\mu$mole melatonin formed per gland per hour)
Unoperated controls	
Dark	71.3 ± 8.6
Light	32.0 ± 2.5*
Blinded (group 1)	
Dark	64.6 ± 6.9
Light	71.6 ± 5.4
Inferior accessory optic tract lesions (group 2)	
Dark	61.6 ± 5.4
Light	50.0 ± 5.5
Optic tract lesions (group 3)	
Dark	76.6 ± 6.6
Light	32.1 ± 4.7*
Inferior accessory optic tract and optic tract lesions (group 4)	
Dark	65.3 ± 10.5
Light	57.5 ± 8.4

* $P < .001$, t test.

Histological appearance of lesions of the medial forebrain bundle was identical to that of lesions described previously (*9*). In each case the medial forebrain bundle was transected in the lateral hypothalamus caudal to the optic chiasm. Lesions were confined, for the most part, to the lateral hypothalamus with only occasional and subtotal involvement of the ipsilateral optic tract. The contralateral tract showed severe glial scarring as a result of the ipsilateral enucleation. Lesions of the optic tract destroyed a large part of the dorsolateral thalamus. The lateral geniculate nuclei, both ventral and dorsal, were totally ablated, and the lesions extended by varying amounts into the adjacent thalamus, subthalamus, pretectum, superior colliculus, and cerebral peduncle. In some instances there was involvement of the hippocampus and cerebral cortex. Each lesion, however, completely transected the optic tract along the cerebral peduncle prior to its entry into the thalamus. A photograph of a representative lesion (group 4) is shown in Fig. 2.

These data demonstrate a specific role for the inferior accessory optic tract in the control of pineal function. With only one inferior accessory optic tract intact, animals are able to maintain a normal pineal HIOMT response to light even when the primary optic tracts and the superior accessory optic tracts have been sectioned. If both inferior accessory optic tracts are cut, however, the animals fail to retain a pineal HIOMT response to continuous light even though the primary retinal projection may be intact. In the pineal, HIOMT participates in the production of melatonin (*1*). Continuous environmental illumination suppresses the activity of HIOMT and the formation of melatonin (*3*). Continuous illumination for short periods also produces, in the mature female rat, an increased incidence of estrus and an increase in gonad weight (*7, 10, 11*). This response to light, like the pineal HIOMT re-

sponse, is abolished by blinding and by superior cervical ganglionectomy (*8*). Since exogenous melatonin inhibits the gonadal effects of constant light (*12*), it is likely that these effects are controlled, at least in part, by the pineal (*8*). This is supported by a recent experiment in which we found that the changes in estrous activity, ovarian weight, and pineal HIOMT activity induced in rats by continuous illumination are also abolished by bilateral lateral hypothalamic lesions that section the inferior accessory optic tracts in the medial forebrain bundle (*13*). These findings appear, then, to establish a function for the inferior accessory optic tracts, separate from that of other retinal projections, in the control of several neuroendocrine responses to environmental light.

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Averaged Neural Electrical Activity and Arousal

Abstract: *Multiple-unit activity from many brain sites of the unrestrained cat was recorded by means of an averaging technique during all phases of sleep and waking. All thalamic and medial reticular sites showed activity levels closely correlated with the animal's observed behavior even under conditions when the electroencephalogram appeared dissociated from behavior.*

Since the description of the ascending reticular activating system by Moruzzi and Magoun (*1*), many investigators have demonstrated that electric stimulation of this system produces behavioral arousal and electroencephalographic desynchronization. On the basis of electric stimulation of the brain it has also been suggested that the thalamic nuclei may regulate the "state of consciousness" (*2*). Several factors of cerebral physiology vary with the level of arousal, including electrocorticograms, d-c potentials, impedance, temperature, and cerebral blood flow (*3*). However, the spontaneous activity of the reticular and thalamic structures has not yet been correlated with the behavior of the awake, unrestrained animal. In this report the relationship between spontaneous neural activity and arousal is investigated.

Data were collected from 26 unanesthetized and unrestrained male and female adult cats. Records were obtained from more than 200 brain sites, including cortical, thalamic, reticular, and lower sensory nuclei, as well as from nonsensory structures. Placements of electrodes were verified histologically. All recordings, whether subcortical or cortical, were made with Teflon-coated stainless steel electrodes led to a Winchester connector plug that was mounted over the animals' frontal sinus. The electrodes were 100 or 200 μ in diameter and they were arranged side by side. The bare tips were separated 0.5 to 1.0 mm. Electromyographic (EMG) activity was recorded by bipolar stainless steel wire electrodes that were implanted into the deep musculature of the neck.

Electric signals generated by both neural and muscular activity were led through a-c coupled amplifiers to an oscilloscope and an "averaging" circuit; this averaging circuit ("integrator") has been used extensively and has been described (*4*). The input was averaged by passing the amplified a-c signal through a full-wave rectifier and a resistor-capacitor circuit smoothing filter with a 3-second time constant. The resulting d-c voltage output, which was displayed by Esterline Angus penwriters, is directly proportional to the amplitude and the frequency of the activity recorded. The band pass of the amplifiers was set at 80 to 10,000 cycle/sec in order to eliminate slow waves in the electroencephalogram (EEG) frequency range and to record only fast activity. This recording of fast activity is derived from multiple units (*5*). The output was expressed in microvolt-milliseconds and was calibrated by passing a 1-msec square wave at 1000 cycle/sec through the circuit.

All recordings were obtained by continuous monitoring of the EEG and EMG; the animals were also observed through a one-way window. The following characteristics were used to define certain behavioral states: (i) arousal (walking, grooming, orienting to external stimuli, and desynchronization of EEG); (ii) quiet alert (sitting or lying, head erect, eyes open, and desynchronization of EEG); (iii) drowsy (head falling, eyes closing, and spindles in EEG); (iv) slow-wave sleep (lying, head down, eyes closed and slow-wave EEG); and (v) paradoxical sleep (lying, intermittent twitching, atonia of neck muscle, and desynchronization of EEG).

The activity recorded from a thalamic site during changing behavioral states is shown in Fig. 1. The multiple-unit activity is indicated by the top trace; the third trace shows the simultaneous average of the multiple-unit activity. There is a marked increase in neural activity at the thalamic recording site during arousal and this is accompanied by a tonic increase in neck EMG activity and a typical EEG desynchronization pattern. Spontaneous rises in thalamic activity often occur several seconds before any overt or EMG signs of arousal. Both multiple-unit and averaged activity at the thalamic site gradually decline as the animal becomes less alert; this activity continues to decrease as spindles appear in the EEG. Averaged thalamic activity reaches its lowest level during slow-wave sleep. In the transition from slow-wave to paradoxical sleep, there is an initially slow, and then rapid, rise in thalamic activity. This