Summer 1969

RESPONSE OF THE RAT OVARY TO CONTINUOUS LIGHT: EFFECTS OF LIGHT FREQUENCY, INTENSITY AND DURATION OF EXPOSURE

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70-9027

LAMBERT, Helen Haynes, 1939-

RESPONSE OF THE RAT OVARY TO CONTINUOUS LIGHT: EFFECTS OF LIGHT FREQUENCY, INTENSITY AND DURATION OF EXPOSURE.

University of New Hampshire, Ph.D., 1969
Physiology

University Microfilms, Inc., Ann Arbor, Michigan

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by

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A THESIS

Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of

Doctor of Philosophy

Graduate School

Department of Zoology

August 1969

This thesis has been examined and approved.

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21 August 1969
Date

ACKNOWLEDGEMENT

I would like to express my deep appreciation to Paul A. Wright for his generous support and encouragement throughout this endeavor. I also want to extend thanks to Stuart Dunn of the Botany Department, and to his students Enola Stevenson and Bill Flahive, for the loan of the thermopile apparatus and instruction in its use; to E. D. Bickford of the Sylvania Company for custom-made fluorescent lamps and the spectral data on Sylvania products; and to Bob Lambert and other members of the Physics Department for technical advice, assistance with wiring, and the loan of the Keithley electrometer.

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ABSTRACT

RESPONSE OF THE RAT OVARY TO CONTINUOUS LIGHT: EFFECTS OF LIGHT FREQUENCY, INTENSITY AND DURATION OF EXPOSURE

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Photoperiodic phenomena among animals are reviewed; the persistent-estrous response of the female rat to continuous light exposure is described and possible neuroendocrine mechanisms in its development are discussed. The question of the receptors responsible for photosexual effects is explored and an experiment proposed to delineate the action spectrum of these effects in the female rat.

Rats exposed to continuous white light at an intensity of 200 microwatts/cm^2 for 8 or 30 weeks experienced a significant advancement in the age of puberty (as evidenced by vaginal introitus), significantly prolonged periods of vaginal estrus, and a significant reduction in ovarian weight, compared to rats exposed to 12 hours per day of the same intensity.

At an intensity of 100 microwatts/cm^2, 8 weeks of continuous white light also produced a significant acceleration of sexual maturity and a significant prolongation of vaginal estrus, as compared to animals receiving 12 hours per

day of the same intensity. By contrast, 100 microwatts/cm^2 of red or green light did not consistently advance puberty, nor did either induce a significant increase in vaginal cornification at 8 weeks of exposure, although some prolongation was observed. At this lower intensity, after 15 weeks of exposure, both red and green continuous light brought about significant increases in estrus, but neither was so effective in this respect as was continuous white light of the same intensity, which by 15 weeks induced an almost continuously estrous state. At 8 weeks of exposure, no significant decreases in ovarian weight were found for continuous white, green or red light at intensities of 100 microwatts/cm^2. But by 15 weeks of exposure, all three continuously lighted groups underwent a significant reduction in ovarian weight as compared to rats under 12 hours of white light at 100 microwatts/cm^2 even after adjustment for differences in body weight among the groups. Again continuous white light was much more effective than continuous red or green, but red and green effects were not significantly different for advancing maturity, inducing estrus or reducing ovarian weight.

Possible explanations for these results are discussed. Measurements of the penetration from the three light sources into various parts of the rat brain in vivo were made with a small photovoltaic cell; a larger percentage of red light than of green or white was found to penetrate to the brain.

SECTION I

INTRODUCTION

1. Historical Background

A. Photoperiodism

The survival of a species in a non-uniform environment requires mechanisms which permit the organism, when necessary, to adjust its most essential biological functions to changes in the surroundings. A great selective advantage is conferred by the ability to utilize information available in the environment to tell "what time it is", and to anticipate periodic changes with the appropriate adjustments. Daily and seasonal variations in natural lighting furnish a reliable timepiece for living things to thus synchronize and schedule their activities.

The diurnal alternation between light and dark, for many organisms, serves to synchronize an internal "biological clock", which has a free-running period of approximately 24 hours under constant lighting conditions. Such "circadian" rhythms have been demonstrated for a variety of life functions and in many living forms. A few examples are luminescence in the marine dinoflagellate Gonyaulax polyhedra (204), leaf movements in the bean plant Phaseolus multiflora (72, 74, 100) and adrenal gland secretions, body temperature and liver glycogen content in the laboratory mouse and in

man (11, 145, 190, 191, 237, 353). There are many indications that the capacity for circadian time-keeping is intracellular (229, 342, 357), and a vast literature deals with the physicochemical basis for these "endogenous self-sustaining oscillations" (306) and with their relation to environmental rhythms, as well as other problems of the fascinating field of biochronometry. Within limits the more or less inherent circadian rhythms of organisms can often be entrained or synchronized to environmental illumination cycles, enabling the organism to stay in phase with its surroundings. Aschoff (8) has coined the term Zeitgeber for the function of photoperiod in such situations; in many instances light is the dominant synchronizer of the basic circadian rhythm.

Related to endogenous circadian cycles and their synchronization to daily-light-dark cycles is the phenomenon of photoperiodism, the control of plant and animal activities by the seasonal variation in day length. Annual changes in natural photoperiod can serve as an extremely accurate calendar for scheduling periodic biological activities at a propitious time of year. The flowering of ragweed and the nesting of sparrows and juncos are conspicuous springtime phenomena prompted by the increasing length of the solar day. It seems plausible that such annual photoperiodic responses involve the same "timepiece" as persistent circadian rhythms, as was first suggested by Bunning (71). According to the Bunning hypothesis, calendar time measurement may be accomplished by comparison of the internal rhythm or

endodiurnal oscillation with the external, seasonally variable rhythm of photoperiodic clues. If the external and internal rhythms are out of phase, a "stress of entrainment" (74) results; if they are in phase, a reinforcement or "beat" phenomenon occurs. Either phase relation might drive a photoperiodic response. A considerable debate (12, 72, 73, 139, 196, 306) has emerged over the relative importance of endogenous diurnal cycles in determining seasonal photoperiodic responses, a question which is complicated by the apparent existence, at least in some birds and mammals, of an endogenous quasi-annual or "circennial" clock as well, which may persist independently of photoperiodic regulation (139, 140).

A variety of animal functions are more or less influenced by photoperiod: the migration of fish (15, 293), diapause and polymorphism in insects and mites (251, 252, 299, 348), molt and pelage in the varying hare (264) and some mustelids (54, 193). But undoubtedly the most prominent photoperiodically regulated functions are reproductive. Among vertebrates, the breeding of many species is affected to some extent by seasonal changes in day length, with other factors contributing to the timing of reproductive activity. Particularly among poikilotherms, temperature assumes a major influence, but photoperiod has been shown to be a significant factor for several species of fishes (15, 199) and some lizards (24, 164).

Photoperiodic control of annual reproductive cycles is most highly developed among birds, especially temperate-zone

migrants. Rowan's (331) pioneering studies on the Slate-colored Junco showed that exposure to long artificial photoperiods in the fall and winter caused the testes of this species to recrudesce from a dormant state. This finding was later confirmed by other workers for many other bird species (55, 76, 136, 138, 378) and shown to be a direct effect of light and not a secondary result of increased activity (51).

Experimental lighting exposure has demonstrated that in many photoperiod-sensitive birds, there is an optimum long daily photoperiod for testicular stimulation; longer periods have no additional effect. The light exposure need not be uninterrupted to be effective (138, 378); in fact a dark period may be necessary (32). Prolonged exposure to natural or artificial long days results in a refractory state, in which the bird is insensitive to continued illumination. This unresponsive condition is terminated after exposure to short photoperiods, such as occur in nature in the fall. The rate of gonadal growth is also a positive function of the intensity of light used, above a fairly low threshold up to a maximum intensity which varies with species (23, 50, 135, 235).

The relative importance of photoperiod in controlling testicular cycles varies even among bird species. The White-crowned Sparrow, Zonotrichia leucophrys gambelii, is an "obligate" photoperiodic species; testicular development does not occur at all under short artificial photoperiods (138, 141). At the other extreme are the Short-tailed Shearwater

Puffinus tenuirostris (270) and the Weaverfinch Quelea quelea (261), for which light merely functions as a quite dispensable frequency monitor for a marked endogenous circennial periodicity of reproduction. Probably most tropical non-migratory birds fall in the latter category (269), and domestic birds such as the duck (38, 39) appear to be intermediate.

Photosexual responses in male birds have been more thoroughly investigated than in females, but many experiments on domestic species (1, 78, 163, 374) have indicated that the ovarian cycle can also be influenced by photoperiod, although behavioral and other stimuli are often of greater importance than in males.

The physiological mechanisms of photoperiodic stimulation in birds have been extensively studied. In the domestic duck, testicular stimulation by light can be prevented by lesions in the supraoptic or paraventricular nuclei of the hypothalamus, or along the neurosecretory tracts from these nuclei to the median eminence, or by permanent interruption of the hypophysial portal system connecting the median eminence to the adenohypophysis (32, 33). In the White-crowned and White-throated Sparrows, photo-activation of the testes is accompanied by increased phosphatase activity and depleted neurosecretory material in the supraoptic nucleus and median eminence (137, 142, 295, 379). From these and other studies (162, 375), it seems that light, acting through the nervous system, stimulates hypothalamic center(s) to greater secretion

of neurohormones which in turn control anterior pituitary secretion of gonadotropic hormones.

Similar hypothalamo-hypophysial mechanisms have been implicated for some mammals whose reproductive season is influenced by photoperiod (112, 208, 302). However, there appear to be no mammals for which photoperiodic control is as "obligate" as in Zonotrichia among the birds. For the mammals which have been studied, light merely provides a Zeitgeber for events which would occur eventually anyway, according to an endogenous circennial timer (9, 125), and are often modified by other environmental factors as well.

Among domestic mammals, some photoperiod-sensitive species, such as the horse (77), are brought into reproductive readiness by long photoperiods, while others, such as sheep and goats (57, 123, 186, 202, 271, 296) respond similarly to short days. Still others, such as cattle and pigs (297), do not seem to be consistently affected by photoperiod. Considerable study has been devoted to increasing fertility in domestic animals by artificial lighting, with variable results (118, 281, 296, 360, 368). Apparently domestication has had an eroding effect on photoperiodic controls in some species, if such controls ever existed.

Similarly among primates there is little indication that photoperiod exerts a major influence on reproduction. A study of the rhesus monkey menstrual cycle (127) showed no alteration with long-day exposure. In the human female, however, one investigator (102) claims to have entrained

ovulation by illumination of the subjects during the night hours, and suggests such a procedure may offer a new "rhythm" method of birth control. Another study reported significant alteration in the human male 17-ketosteroid excretion pattern under continuous white, red or blue light (216).

In non-domestic, seasonally breeding mammals, photoperiodic control of reproduction persists with greater efficacy. Light-induced alterations in breeding have been demonstrated in the raccoon Procyon lotor (58), and the mink Mustela vison (192). Undoubtedly the most extensively investigated photoperiodic phenomenon among nondomestic mammals has been the control of estrus in the ferret, Putorius vulgaris. This mustelid, a long-day mammal, normally mates in spring, but reproductive readiness can be accelerated in both sexes by exposure to long photoperiods in winter (53). This acceleration requires the retina (361) and intact optic nerves (56). However, even blinded ferrets will come into estrus, although often later than usual (362, 363), suggesting an endogenous rhythm.

For the ferret, the dark period is important as well as the light (193, 195, 203); continuous illumination is less effective in accelerating estrus than 14 to 16 hours of light per day (194). Long-day stimulation in summer or autumn inhibits the development of estrus, sometimes for years (110, 111), suggesting a seasonal variation in sensitivity similar to the late-summer refractory period in birds. Studies on intensity have been equivocal (195, 272, 276), but the level

of illumination appears to be much less important than its duration.

The neuroendocrine mechanisms of estrus induction by light in the ferret are, at least in many respects, similar to those for testicular activation in birds. Section of the pituitary stalk, including permanent interruption of the adenohypophysial portal system, prevents the photoperiodic response, as does hypophysectomy or blinding (56, 112). Hypothalamic lesions can induce estrus in the ferret in mid-winter (114), indicating that the endogenous timer, as well as the photoperiodic response, involves this part of the brain. The nervous connections from the eye involved in the response are less well understood; the classical visual pathways do not appear to be involved (82). Several workers have found that removal of the superior cervical ganglia of the sympathetic chain interferes with photoperiodically induced estrus (2, 113) in the ferret. More recently it has been demonstrated that this effect of sympathectomy is not a result of the reduced light reaching the retina because of the ptosis which follows this surgery (275). With the apparent involvement of the sympathetic nervous system in photosexual responses, the nervous pathway from retina to hypothalamus in the ferret becomes even more elusive.

Among seasonally breeding rodents, some degree of photoperiodic control has been shown to exist in Peromyscus leucopus (372), Microtus agrestis (17, 83) and Microtus arvalis (277, 360). More interesting from an evolutionary

standpoint is the influence of lighting conditions on sexual function in several polyestrous rodents which have a continuous reproductive season, at least in the laboratory. The rhythm of diurnal lighting is important in the control of ovulation and the estrous cycle in mice (63, 184, 224), hamsters (5) and rats (66, 132, 133, 206, 260). Exposure to continuous illumination accelerates sexual maturation in the female rat (148, 220, 263), while housing in constant darkness or blinding retards maturity (147, 364). Prolonged constant light induces persistent vaginal estrus in female rats (66, 101, 148, 206, 220) and hamsters (234), but not in mice (59, 184, 223), while constant darkness has been variously reported to have no effect on the estrous cycle (66) and to result in a lengthening of diestrous periods (148, 220) in the rat. Long photoperiods alone will not bring about a persistent estrous state; rats kept outdoors during summer have normal estrous cycles (66). The physiological mechanisms controlling gonadal function are profoundly affected by light even in these laboratory species; in extreme conditions such as continuous light or dark, photic influences can be more powerful than is implied by their normal role for mammals as a synchronizer of reproductive function.

B. Light Estrus in the Rat

Since persistent vaginal estrus induced by continuous illumination was first described in the rat (66, 206), it has been the subject of many investigations. It is of interest

partly because it furnishes a classic, if extreme, example of neuroendocrine response to an environmental stimulus well-known to be a dominant synchronizer of physiological cycles. However, much of the experimental work on "light estrus" has also been fruitful in terms of understanding, through study of this pathological condition, the mechanisms involved in the control of pituitary-ovarian function in the normal cyclic rat.

Typically, the female rat exposed to continuous illumination from birth or weaning matures early, has a few regular estrous cycles, and then exhibits irregular and longer cycles, with increasing periods of vaginal cornification and behavioral estrus, and after long exposure to light enters a stage of more or less constant estrus (66, 148, 206). In Fiske's original study (148) she found the pituitaries of such rats, even when castrated, had a higher follicle-stimulating hormone (FSH) activity, but a lower luteinizing-hormone (in the rat, more properly, ovulating hormone, or OH) activity, than the pituitaries of rats housed in constant darkness. Light's effect on gonadotropin content was correlated with histological changes in the pituitary (308) and with marked stimulation of the ovaries (308) and reproductive tract in light-exposed animals, quite similar to the changes seen with photoperiodic stimulation of seasonally breeding mammals. In the early stages of constant light exposure, the ovaries are large, with both follicles and corpora lutea, but after many months of light, the ovaries become nearly atrophic: small, follicular, often cystic and devoid of corpora lutea

(148, 308, 364). At the same time the pituitary content of FSH becomes reduced (148).

These first studies of light estrus implied that light, presumably acting through the nervous system, stimulates the pituitary's synthesis and/or release of FSH, but inhibits that of OH. This conclusion has been borne out by more recent studies of pituitary gonadotropic hormone content using newer bioassay procedures. Although there is some variation (probably due to differences in intensity and length of exposure (248, 260) and in age and strain of rats used (128)) the FSH content of pituitaries in rats exposed to constant light is consistently found to be as high as, or higher than, that of rats under normal light-dark alternation, while the OH content under light is lower than the estrous values of cyclic rats (64, 247, 268, 303, 346).

In the first weeks of continuous light exposure, the inhibition of OH is not sufficient to prevent ovulation; in fact OH may initially be elevated (248). But in time the cyclic surge of OH release needed for follicular rupture (340, 341) is somehow blocked by light. The appearance of the ovaries at this time, with large follicles and normal interstitial tissue (165, 166) indicates that at least some OH, as well as large amounts of FSH, is being released by the pituitary, although apparently not enough to trigger ovulation. Vesicular follicles of ovulatory size are definitely present, although some metabolic studies indicate that the hyperplastic or cystic follicles are not functionally the same

as normal preovulatory follicles (65, 371). Persistent estrus rats can be induced to ovulate by injection of human chorionic gonadotropin or progesterone (346) or by neural stimuli such as coitus (101, 222), progesterone treatment can also increase the OH content of the light-treated rat's pituitary (268).

Some OH secretion is thought to be necessary for the production of estrogen by the follicles (146, 182) which is certainly occurring in rats with persistent vaginal cornification. The high estrogen levels evidently produced by the light-treated ovaries would be expected to inhibit the secretion of FSH by the anterior pituitary, probably via the hypothalamus (158,161). Such a negative feedback might explain the eventual atrophic state of the ovaries in light-treated rats, but does not explain the persistence of FSH secretion for many weeks in the face of high estrogen titers. Related to feedback are the apparently contradictory findings that (1) compensatory ovarian hypertrophy after unilateral castration is lessened under continuous light (91) but (2) bilateral castration causes an increase in pituitary FSH and OH which is not altered by continuous light exposure (346). Clearly the alterations that continuous light brings about in the steroid-gonadotropin feedback system are complex.

Jochle (222) has suggested that light acts on the neuroendocrine system by raising the hypothalamic threshold to steroid feedback, perhaps by abolishing a rhythm of sensitivity normally present in females but not in males, and

instituting an acyclic male pattern of gonadotropin secretion. In an extensive chronological study of light-induced persistent estrus, Lawton and Schwartz (248) observed a marked asynchronism in pituitary, ovarian and uterine functional rhythms in the early stages of light treatment, and also concluded that the effect of light is primarily on a cyclic component of gonadotropin release control, presumably located in the hypothalamus.

Much evidence suggests that light stimulates abnormal levels of FSH and OH by exciting, or disinhibiting, the hypothalamic neurosecretory mechanism which normally regulates gonadotropic hormone secretion. Light-induced alterations in the neurosecretory structures of the hypothalamus have been reported: increased activity and enlargement of neurons in the supraoptic nucleus, and accumulation of neurosecretory (aldehyde-fuchsin positive) material in the supraoptico-hypophysial tract and median eminence (151, 154, 155). The hypothalamic concentration of FSH-releasing factor has been found higher after 21 days of constant light, even though pituitary FSH was not yet elevated (290). In fact, continuous light is reported to stimulate the ovaries of hypophysectomized animals with transplanted pituitaries (303), suggesting that the releasing factor may travel via the blood stream in such animals.

Like continuous light, certain hypothalamic lesions can induce precocious puberty (62, 115, 116, 173), persistent vaginal estrus, and small follicular ovaries lacking in

corpora lutea (209, 241, 358, 366). Alterations in pituitary FSH and OH content in these lesioned animals have been demonstrated (95, 116, 358), although it remains unclear whether the primary effect of the lesion is on synthesis or release of gonadotropin, or both. Similar to light-treated persistent estrus rats, rats with hypothalamic lesions show no compensatory ovarian hypertrophy after unilateral castration, if the lesion is in the anterior hypothalamus (95, 160). Also, such lesioned rats can be made to ovulate with progesterone if the preoptic-suprachiasmatic region is intact (183). The parallels between the two persistent estrous conditions imply that, at least in some respects, exposure to constant light is equivalent to destroying part of the brain.

Still another syndrome of persistent estrus, with ovulatory failure and micropolycystic ovaries, is produced by treating neonatal female rats with testosterone (18, 180, 200). The pituitaries of such animals after puberty contain low to normal amounts of total gonadotropin, but very low concentrations of OH (280). Yet these pituitaries, if transplanted into normal hypophysectomized animals are capable of normal secretion (343). Progesterone administration in the intact testosterone-sterilized rat can increase pituitary OH content (177), and electric stimulation of the arcuate-ventromedial area of the hypothalamus in such progesterone-primed animals can induce ovulation (19, 178). It would appear that exposure to exogenous androgens in infancy permanently alters the pattern of gonadotropin secretion

determined by the hypothalamus; a similar effect of endogenous testicular secretion is thought to be responsible for the development of male secretion patterns in the normal neonatal male rat (175, 176, 200, 369).

Similarities among these three kinds of persistent estrus rats: those in constant light, with hypothalamic lesions, and with testosterone treatment, naturally lead to the hypothesis that each syndrome involves the same functional lesion in the hypothalamic gonadotropin-release-regulating system, i.e., an interference with the cyclic pattern of release characteristic of the female. Some reports indicate, however, that although the presenting symptoms may be similar, the etiology of the three persistent estrous syndromes may not be identical. For instance, ovarian compensatory hypertrophy is normal in the testosterone-treated rat (19), but not in the constant light rat or the rat with hypothalamic lesions (91, 95, 160). The inducement of precocious puberty by continuous light was found to be independent of the similar effects of anterior hypothalamic lesions (325). Neonatal testosterone treatment can prevent the usual pituitary and ovarian response to constant light exposure (85), suggesting that the masculinization of the hypothalamus renders it unresponsive to light. Similarly, small lesions in the suprachiasmatic region interfere with the usual estrous response to light (91). These results do not reveal whether the three syndromes are functionally the same, but they emphasize that all three involve some malfunction of the hypothalamic gonadotropin-release control mechanism.

Many recent reports indicate that in normal cyclic female rats there is a dual regulation of adenophypophysial gonadotropin secretion. A basal or tonic control level is associated with the ventromedial-arcuate complex of the medial basal hypothalamus - the "hypophyseotropic area" or HTA - and is the immediate source of the neurohumoral releasing factors which travel to the adenohypophysis via the hypophysial portal system. The HTA level is relatively autonomous in that it is not blocked by barbiturate treatment (250), and the HTA continues to initiate gonadotropin production and release at a basal level even after complete deafferentation (187, 188). The HTA seems also to be capable of some steroid feedback responses even when severed from the rest of the brain (187), although the steroid threshold is very different from that which obtains when afferent connections are intact. But then the anterior pituitary itself is capable of some feedback responses to steroid implants (61), and blood steroid levels (80), and there may even be internal feedback circuits between the HTA and the anterior pituitary (88). This basal level of gonadotropin control may in fact be a multiple, local feedback system.

By contrast the normal cyclic changes in gonadotropin secretion associated with ovulation are regulated by barbituate-sensitive (250) neural mechanisms which reside outside the HTA but act through it and its releasing factors on the adenohypophysis (187). The anterior hypothalamic, supraoptic and preoptic area seems a likely candidate as a location

for this cyclic release center. Electrical stimulation of this area can induce ovulation in the anesthetized rat (90, 129), as in the light estrous rat (70), and it is this area in which lesions abolish ovulation and induce the persistent estrous state described above. Furthermore this area of the hypothalamus is sensitive to estrogen (161, 347) and progesterone (21).

It seems likely that constant light exerts its effects on the female rat through this higher control center, deranging its cyclicity of function. The observations of enhanced neurosecretory activity in the supraoptic region (151, 154, 155) after light exposure would support this anatomical location as part of the pathway involved in constant light's effects. Small lesions in the supraoptic area prevent the usual response to light (97). It is of interest that responses to visual stimulation have been recorded electrically in this area of the brain (93, 144), and that such responses are affected by estrogen treatment (256). However, electrical responses to other types of stimuli e.g. manipulation of the cervix, can also be recorded from this area, which is apparently involved in sexual behavior (201) as well, and these responses are also affected by estrogen (93) and progesterone (94).

Many different external stimuli can contribute to the timing of ovulation. In rats in which the normal release of OH has been blocked by barbiturates or chlorpromazine, ovulation can be triggered by coitus or electrical stimulation of

the cervix (131, 197, 198, 394). Probably light is normally involved in the timing of cyclic OH release. Everett and Sawyer originally (132, 133) postulated the existence of a diurnally excitable neural center which, in response to steroids (130, 339), initiated the ovulatory surge of OH. In studies with the barbiturate blockade of ovulation, Everett (132) has found that the critical period for blockade (2 PM on the proestrous day) recurred at the same time on succeeding days, so that ovulation could be repeatedly postponed by drug administration at this hour, with the persistence of the same ripe follicles. It seems possible that continuous light removes this (perhaps light-clued) diurnal excitability, resulting in overlapping generations of follicles.

A possible factor in the rat's response to constant light is the pineal body. The function of this small structure in birds and mammals, where it does not appear to be photoreceptive (227, 228, 316), remains poorly understood, but the pineal has long been associated clinically with abnormalities of sexual development (236). A large number of recent studies suggest that the pineal may be involved in light-induced gonadal changes in rodents.

Although there is very little evidence that the pineal is photoreceptive in rodents, it is profoundly affected by lighting conditions. The pineals of Peromyscus and of the rat undergo structural changes when the animal is in constant light or dark (310, 311, 330). A significant decrease in pineal weight is found in male or female rats exposed to constant light, even if they are hypophysectomized or

gonadectomized (152, 153). A demonstrated diurnal rhythm of rat pineal norepinephrine content is abolished by blinding or exposure to constant light or dark (391). A diurnal rhythm is also found in the pineal content of serotonin (5-hydroxy-tryptamine) (312, 352) in the rat; this amine is higher during the light hours. The serotonin rhythm is also abolished by blinding (314) or continuous light, but not by continuous dark (352), and can be altered by altering lighting cycles (351). In the neonatal rat, the diurnal serotonin rhythm can be altered by extra lighting exposure even when the animals are blinded (395), suggesting that extraretinal receptors may be involved. In the adult rat, the rhythm of serotonin (149) and the effects of light on the enzyme which forms it, 5-hydroxytryptophan decarboyxlase (98, 349), can be abolished by superior cervical ganglionectomy, which also abolishes the rat's, and the ferret's, estrous response to light (2, 13, 385). The superior cervical ganglion is the source of the primary nerve supply of the pineal gland (228), so the operation is equivalent to denervating it.

Another diurnal rhythm is observed in the rat pineal content of melatonin (5-methoxy-N-acetyl tryptamine) (313), and of hydroxyindole-O-methyl transferase (HIOMT), an enzyme required for the formation of melatonin from serotonin (14). Like norepinephrine, melatonin and its enzyme are highest in the dark hours. In the rat (286, 287, 389), and in the hamster (6), continuous darkness or blinding brings about an increase in pineal HIOMT, while constant light causes a

decrease. These effects of light on the pineal require the eyes, but not, apparently, the primary optic tracts (286). The inferior optic tracts, which leave the optic nerves at the chiasm and run in the medial forebrain bundle to the midbrain tegmentum, are claimed to be necessary. As with the effects of light on pineal serotonin content, the light-induced variations in melatonin and HIOMT can be prevented by superior cervical ganglionectomy, even when the ptosis resulting from the surgery is compensated by removal of the eyelids (385). That the changes in these substances in the pineal depend on the sympathetic innervation of this organ was shown by an experiment (393) in which the pineal was transplanted to the anterior chamber of the eye; in this location the levels of these substances were normal but were not affected by lighting conditions. The HIOMT content of the pineal <u>in situ</u> can be decreased by electrical stimulation of the preganglionic trunk to the superior cervical ganglion (68). Thus the mediation of the effects of light on the pineal would appear to be nervous rather than hormonal; this is consistent with the fact that gonadectomy and hypophysectomy do not interfere with the pineal weight reduction (153) and HIOMT decrease (387) in constant light.

That the pineal is severely affected by light exposure seems well established; that it somehow mediates the gonadal response to light is more difficult to prove. Certain kinds of pineal tumors have long been associated with precocious puberty (84, 228, 236) leading to the hypothesis that the

pineal's function is concerned with the alterations in pituitary-gonadal function which mark the attainment of sexual maturity. Most frequently the pineal's role has been supposed to be an inhibitory one. Pinealectomy in immature male rats causes an earlier development of copulatory behavior (25) and an increase in gonad and accessory weight (289, 359). In female rats, pinealectomy results in vaginal and ovarian conditions similar to those seen in early continuous light exposure; these effects can be reversed by the administration of pineal extract (392). From these and other studies it would appear that removal of the pineal releases the gonads from an inhibition; perhaps light exposure inhibits the pineal, and depressing pineal function may have the effect of stimulating the gonads. Such an hypothesis is supported by the finding that the administration of pineal extracts interferes with the rat's vaginal (214, 221) and ovarian (392) but not uterine responses to continuous light.

The removal of light stimulation seems to stimulate (or disinhibit) the pineal and allow it to depress gonadal function. Blinding male or female hamsters or rats causes involution of the gonads and accessories, but this result of blinding can be prevented by pinealectomy (321, 322, 323) or by superior cervical ganglionectomy (318, 319, 320). Continuous dark or very short photoperiods (1 hour/day) also cause atrophy of the testes in male hamsters, and can prevent compensatory hypertrophy after unilateral castration (210); these effects of light deprivation can be ameliorated by

pinealectomy. Some studies indicate that rats must initially be exposed to light-dark alternation before their pineals can mediate the depression of gonadal function seen in continuous dark (324, 326).

Wurtman believes the pineal's inhibition of the gonads is due to melatonin, which has been shown to decrease in the light-exposed pineal, and to vary with the normal estrous cycle (386). Administration of melatonin to normal rats delays puberty (289), decreases vaginal estrus and ovarian weight (289, 355, 383) and counteracts the stimulation of the ovaries in pinealectomized animals or those exposed to constant light (81, 384). Some workers have not been able to confirm all of these effects of melatonin (4, 124).

Meltatonin does have a variety of physiological effects. It is a potent lightener of amphibian melanophores, and its injection causes an abrupt fall in the melanocyte-stimulating hormone of the rat pituitary (230). Melatonin is found in the pineal of many mammals (255), and the HIOMT required for its synthesis appears to occur only in the pineal (13). Intraperitoneal injection of melatonin causes an increase in brain serotonin (7); exogenous melatonin can also prevent the diurnal rhythm of pineal serotonin if it is administered during the light period (150). Melatonin injection or implant of melatonin in the hypothalamus or midbrain decrease the pituitary content of OH (169, 289) and reduce plasma levels of OH as well (168). Exogenous radioactive-labelled melatonin, on injection, is concentrated by the

pineal, brain and ovaries (390). These many results do not point to a simple explanation, but it seems possible that melatonin could act on neuroendocrine centers in the brain which control cyclic functions such as the estrous cycle.

Since the effects of constant light on the pineal appear to be mediated by the same sympathetic pathways that mediate the estrous response to continuous light, it is difficult to determine whether these are parallel effects or links in a causal chain. That the light-induced pineal alterations occur in the absence of the ovaries is definitely known, but the ovarian response to constant light is approximately duplicated by pinealectomy (and they are not additive (392)), so it is difficult to test whether the gonad alterations occur in the absence of the pineal. Hence the pineal remains a puzzling but possibly important element in the rat's response to continuous light.

C. The Question of Receptors

Ocular Receptors. It appears to have been adequately demonstrated that in the rat (66, 97), as in the ferret (55, 56, 82, 273) the eyes are essential for the effects of light on the reproductive system. Whether rods, cones, or neither are the receptors has not been determined. Scharrer (336) long ago suggested that the receptors and neural pathways involved in photoperiodic and other autonomic responses to light were different from those for vision and optic reflexes. Thomson (361) found in the ferret that the ganglion layer of

the retina was specifically necessary for early estrus to occur (the lenses and humors being dispensable), and has postulated that some interaction between rods and cones may be important in the sexual response to light. According to Donovan (110), Thomson believes that photosexual effects occur only in animals with both rods and cones. It is often stated that the "neurovegetative" cells described in the ganglion layer of the mammalian retina by Becher (27, 28) may be the photosexual receptors. This notion is strengthened by the finding that the spectral response for photosexual effects is different from that for perceptual vision.

Careful studies have been done on the visual sensitivity of the duck, Anas platyrhynchos, which for sensory reception and the pupillary reflex has a maximum in the yellow region of the visible spectrum (40). By contrast the most effective wavelengths for testicular stimulation in the duck are the orange and red ones (6000-7500 Å) (40, 46, 47). Similar effectiveness of red light has been reported for the Starling (52, 75), English Sparrow (327) and the Japanese Quail (380). It was recently confirmed in six other species of birds, using equalized intensities of light (338). Shorter wavelengths, even those most effective for visual sensation, are quite ineffective in stimulating the gonads (46, 47, 48).

No comparable study exists on the spectral response of photosexuality in a mammal: Marshall and Bowden's early reports (273, 274) on early estrus in the ferret showed no differences across the visible spectrum, while ultraviolet was

quite effective, but the intensities they used were not equivalent. Ott (298) found that the addition of ultraviolet (2900-4000 Å ngstroms) to artificial lighting [making it more like sunlight] markedly increases the fertility of rats. But there is little firm evidence concerning the action spectrum or the receptors for photosexual responses in a mammal.

The afferent pathways for photosexual effects are quite definitely different from those for perceptual vision, at least in the rat and ferret. Section of the optic nerves (66, 97), like optic enucleation, abolishes the estrous response to light, but the pathway from the optic chiasma to the demonstrated effects in the hypothalamus is far from clear. In ferrets the usual response to extra light occurs after removal of the superior colliculi or interruption of the classical visual projections to the midbrain reflex center and to the lateral geniculate bodies for relay to the visual cortex (82). In the ferret there appear to be no accessory optic tracts (219). In rats, bilateral transection of the optic tracts immediately behind the chiasma, presumably interrupting all central visual projections, did not interfere with the estrous response to constant light (97). More recently Wurtman (388) has reported that lesions in the medial forebrain bundles, which are reported (205, 286) to contain fibers from the inferior accessory optic tracts, do block the rat's estrous response to light. However, these medial forebrain bundles terminate in the midbrain

tegmentum (205, 286), and another report (301) shows that normal ovulation, at least, can occur after extensive destruction of the tegmentum. Thus it is difficult to imagine how photic information usually makes its way to the hypothalmus.

Many histological (60, 205, 239, 242, 307) and electrophysiological (144, 279) searches have been made for direct retinohypothalamic connections in various animals. Some authors have described fibers from the retina leaving the optic nerves just above the chiasma and terminating in various hypothalamic nuclei (60, 239, 267); but others were not able to confirm these observations, so precise anatomical demonstration of such connections, at least in the rat, has yet to be made.

The elusiveness of direct retinohypothalamic nervous connections is made more interesting by the possible involvement of the sympathetic system in photosexual responses. As mentioned above, in the rat (385) and ferret (2, 113), superior cervical ganglionectomy abolishes the normal estrous response to light. With the possible involvement also of the midbrain and of the pineal gland, it seems unlikely that a direct neural link between the optic chiasma and its close neighbor, the hypothalamus, will turn up as the afferent route for photosexual responses.

Non-ocular Receptors. Benoit and his coworkers (for review see (32, 34, 37)) have unequivocally shown that in the duck, encephalic as well as ocular receptors operate in

photostimulation of the testes. In this bird, gonadal activation at high intensities of light can occur even after optic enucleation (30) or transection of the optic nerves (35), and even small amounts of light, when conducted directly to the anterior hypothalamus or the rhinencephalon via a quartz rod, can be an effective stimulus (31, 42). The light sensitivity of the uncovered hypothalamus is reported to be greater than that of the eye for producing testicular development (36) and to extend to all visible wavelengths (46, 47).

However, in the intact duck, only the longer wavelengths apparently penetrate the skull and feathers (44, 45). In a study of the penetrability of the duck's head as a function of wavelength, Benoit found that short wavelengths were poorly transmitted (1/2000 for indigo, 4360 Ångstroms; 1/630 for green, 5460 Ångstroms), but longer wavelengths penetrated much better (1/130 for orange, 6170 Ångstroms; 1/55 for red, 6470 Ångstroms). As much or more light penetrated through the top and sides of the skull as through the walls of the orbit in white ducks, while in dark-feathered ducks with the same eye pigmentation, most of the light came through the thin walls of the orbit (45).

Schildmacher (338) supposes that these differences in penetration may explain the apparent effectiveness of red and orange wavelengths in photostimulation of birds, a difficult point to prove. It is nearly impossible to isolate the effects of the optic receptors from those of the encephalic ones (although the converse can easily be done in blinded animals)

because of the penetration through the orbit when the eye is illuminated. Benoit (34), from experiments with intact ducks at intensities he believes are too low to activate the encephalic receptors, maintains that the retinal sensitivity alone has a peak at 6470 Ångstroms while Hollwich and Tilgner (213) found at similarly low intensities the most effective wavelength was even further in the red, at 7070 Ångstroms.

Benoit's demonstration of encephalic photosensitivity was recently confirmed in the House Sparrow, in which activity cycles can be entrained by light cycles even after removal of the eyes, and of the pineal (282). The evidently direct response of the brain to light in the absence of specialized photoreceptors is not such a startling finding as it might at first appear, although it is in curious contrast to the apparent necessity of the eyes for photosexual effects in rats and ferrets. The lateral eyes of vertebrates are embryologically derived from the diencephalon, as are the functional pineal, parapineal and parietal eyes and frontal organs of certain lower vertebrates; hence, the neurons of this brain region have photoreception in the family. It has been pointed out that (337) neurosecretory cells resemble the rods of the retina in that they have cilia with the 9:0 fibril arrangement.

Whether all cells, or only specialized ones, have the potential capacity to record photic stimuli is an interesting question. The widespread occurrence of phototaxis among unicellular, usually photosynthetic, forms illustrates that a

cell's capacity to respond to light does not depend on the highly specialized lamellar structures of vertebrate rods and cones, although some photopigment must be required. The pigmented muscle fibers of the pupillary sphincter in teleost fishes and some amphibia (344, 345) contract on exposure to light even when denervated. Melanophores of Xenopus larvae respond to illumination when isolated from nervous and hormonal influences (16) and even when grown in tissue culture (294). The smooth muscle of mammalian skin arterioles can contract in direct response to light (170), and excised pieces of skin from a number of vertebrates generate measurable electrical responses to illumination (29).

Nerve cells especially have been found to be photosensitive. Single neurons from several invertebrates have photoreceptive properties: the abdominal ganglion of the crayfish (233, 309), the visceral ganglion of Aplysia (79), and the pallial nerve of the surf clam Spisula solidissimus (232) contain cells from which primary on-off responses to illumination have been recorded. Some interesting work has been done on the action spectrum of these invertebrate neurons (69, 79, 232); it seems likely that both carotenoids and hemoproteins are involved as photopigments.

Photosensitivity of some parts of the brain has been observed among insects (104, 253, 373). Reactions to light were long ago shown (367) and recently conformed (99, 335) in the blinded minnow Phoxinus phoxinus, a property which Scharrer (336) ascribed to the supraoptic region. In

Rana temporaria, pituitary melanophoretic hormone is formed in response to light exposure after removal of the eyes (328). Some of these observations are difficult to interpret because of the subsequent demonstration of photoreceptive structure and function in the pineal or parietal organs of a number of lower vertebrates (105, 107, 108, 120, 121, 122, 231, 284). Some of the responses which have been attributed to brain photoreception may in fact have been pineal responses. Since all available evidence indicates that the pineal is not photoreceptive in birds, this difficulty of interpretation does not arise with Benoit's work on the duck's encephalic receptors.

There are a few indications of extraretinal photoreception in mammals in the literature. The responses of blinded neonatal rat's serotonin rhythm to added light (395) was mentioned previously; the skin and skull of newborn rats is remarkably transparent. An experiment similar to Benoit's quartz-rod investigation was performed by Lisk and Kannwischer (257) on the female rat, using chronically implanted optic fibers to conduct constant light directly to various regions of the hypothalamus in blinded animals. When the fibers were placed in the suprachiasmatic region of blind rats, an estrus response similar to that of seeing animals [with covered optic fiber implants] was obtained, although ovarian weight and ovulation did not decrease as much with the hypothalamic as with the optic route of light. By contrast, when light was directed via a fiber to the arcuate region, fewer cornified smears, an increase in pituitary and ovarian weight, and many

corpora lutea resulted. These findings indicate that in the rat, as in the duck, light reaching the hypothalamus directly can have a profound effect on pituitary-gonadal function.

Whether such effects occur in the intact rat remains to be demonstrated. The photoelectric measurements of Ganong (171) and van Brunt (365) indicate that appreciable amounts of light do penetrate the skulls of several mammals, including the rat, under ordinary lighting conditions. Apparently for the intensities used by most investigators, the amount penetrating is not enough to cause estrous effects in the absence of the eyes, since blinding has uniformly been reported to abolish the response. However, Flament-Durand (154) noted an increase in the size and activity of the neurons in the supra-optic nucleus under constant light even in blinded animals, even though they did not exhibit estrus. It seems possible that high intensities, or more penetrating frequencies, might affect the hypothalamus in the rat enough to bring about an estrous response even without the eyes.

2. Proposed Investigation

The experiments to be described herein were undertaken to determine the action spectrum of light estrus in the rat. Because this phenomenon is a statistical and long-term effect, a large area illuminated for many weeks was required; the use of monochromatic light was deemed impractical. Instead, two spectrally distinct sources, red and green, were chosen from available fluorescent tubes, and compared with white light of

the same intensity. The green source was near the peak of visual sensitivity; the red source, chosen because of Benoit's work, was mostly beyond the absorption range of the rat retina (167, 370). Preliminary experiments with continuous white light were designed to determine the optimum intensity and duration of exposure. The chronology of persistent estrus under the conditions to be used was followed, since the phenomenon is a progressive one. Previous studies have shown wide variation in estrus response to continuous light among various strains of rats (128, 222, 268) at various ages (128, 148, 222, 260), and with different intensities of light (260). Both natural and artificial alternating light-dark conditions were included as controls, since there are few data available on the effects of artificial light per se. It seemed desirable also to determine how much light could penetrate the skull of the rat at the intensities used, and particularly to compare the light sources employed in this respect.

SECTION II

MATERIALS AND METHODS

Female weanling (23-24 days of age) albino rats of the Sprague-Dawley strain, obtained from the Charles River Breeding Laboratories, Arlington, Massachusetts, were used for all experiments. Earlier experiments were conducted in 1964 with animals from the supplier's "SD" (Sprague-Dawley strain) colony, which was then unfortunately discontinued, so that "CD" (Caesarean-derived from Sprague-Dawley stock) colony animals were used for subsequent series.

Animals were placed in the experimental housing immediately upon their arrival, and given 24 hours to recover from shipment before the experimental lighting was turned on. Bedding and nesting material were not provided, in order to maximize exposure to light. Purina rat chow and tap water were given ad libitum throughout the experiments. Body weights were recorded weekly. All rats were examined daily for vaginal introitus, and vaginal smears were made daily after maturation had occurred. Smears were taken by saline lavage, air-dried and stained with aqueous methylene blue or with Harris hematoxylin and Shorr's Single Stain for keratin (185), then classified according to the stages of Long and Evans (262).

At the conclusion of each experimental period, the rats were sacrificed with ether, body weights determined, and the ovaries immediately removed. Ovaries were quickly cleaned of fat and oviduct under a binocular dissecting microscope, weighed to the nearest 0.1 mg. on a Roller-Smith balance, and fixed in Bouin's fluid. Five micron sections were stained with hematoxylin and eosin, or with Greenstein's Five-Dye stain (185).

Experimental lighting was provided by fluorescent fixtures mounted in specially constructed lightproof cabinets (Figure 1), wherein the rats were housed. All operations which required opening the cabinets of the red- or green-exposed groups were carried out with the surrounding room completely darkened. Thus all other light sources were excluded for the duration of the experiment. Cages were rotated within each cabinet at weekly intervals to counteract any effect of different intensities in different parts of the cabinet.

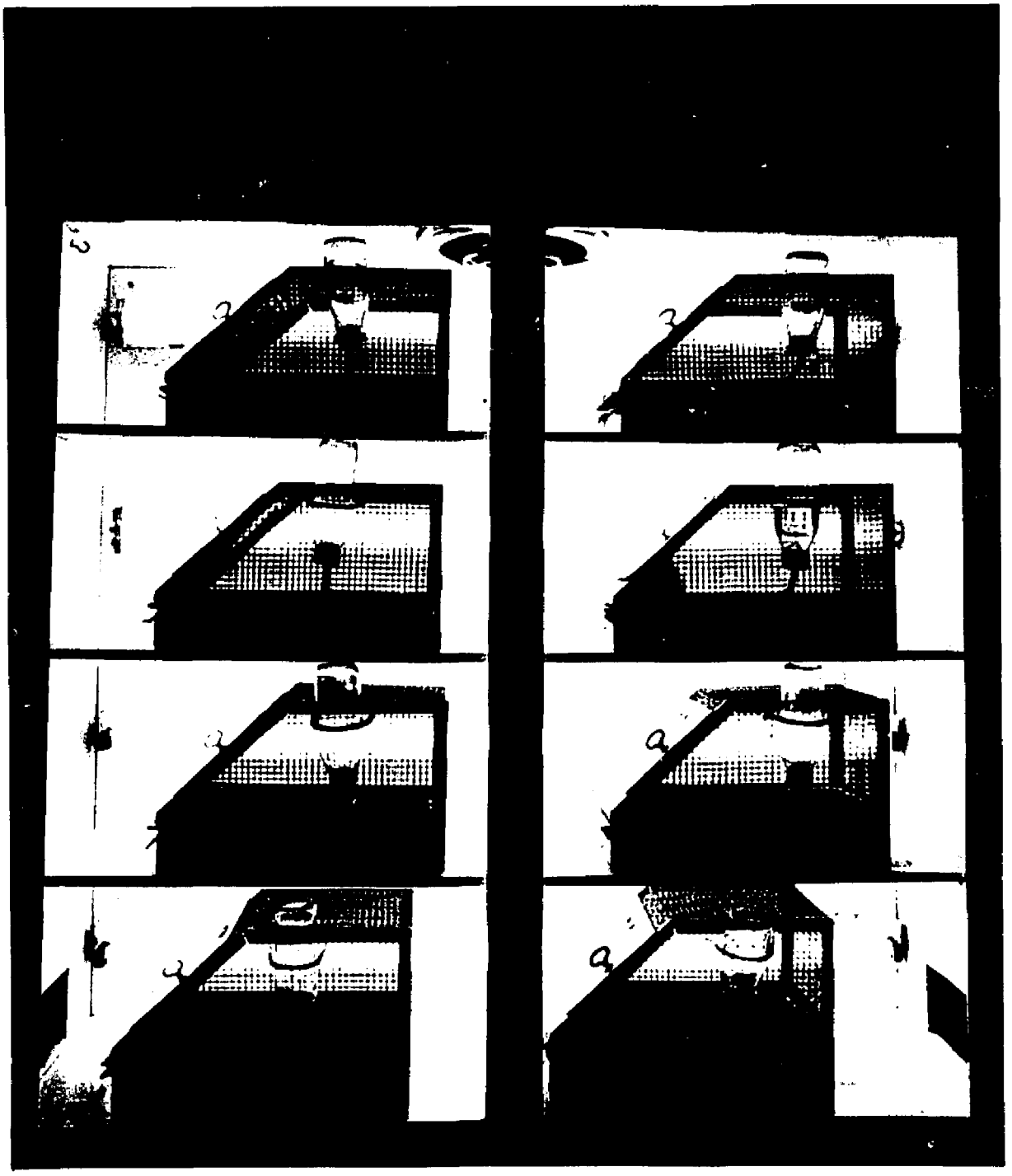
The twelve-hour photoperiod of artificial light was run from 6 AM to 6 PM, controlled by an electric timer (Model APT-10, "Ti-Mite", Paragon Electric Company). The dark period was completely dark for the animals on this 12-hour regimen; the cabinets were sealed during the night interval. "Control" lighting was that prevailing outside the cabinets in the animal room: daylight from a window, with irregular supplements of artificial (incandescent) light, according to the use of the room by other personnel.

Heat in the cabinets was reduced by mounting the ballast of the light fixtures outside the cabinets, and by an exhaust fan, run continuously, in the top of each cabinet. Air was admitted through side vents equipped with light baffles. Temperature in the cabinets, monitored by a recording thermometer, varied from 76° to 80° F., except for a few days in July 1968, when the air-conditioning of the room was overloaded; a maximum of 90° F. was recorded at that time, in both control and experimental groups. The diurnal variation in temperature, derived largely from the central heating of the building, was slightly greater in excursion for the 12-hour photoperiod housing, as might be expected: a range of 4° F., as compared to 2° or 3° F. for the continuously lighted cabinets.

The fluorescent tubes used were Sylvania 40-watt Cool White, Green (both commercially obtained) and custom-made high-intensity red magnesium germanate lamps, also from Sylvania. Spectral energy distribution of the three light sources appear in Figures 2, 3, and 4, by courtesy of the Sylvania Company. It will be noted from superposition of Figures 3 and 4 that the red and green frequency distributions are not quite mutually exclusive. The overlap of the two curves around 6000 Ångstroms is less than 3% of the total energy output of each lamp, as calculated from wattage tables supplied by the manufacturer. Each source also unavoidably induces the mercury resonance lines in the violet and near-ultraviolet range. The spectral energy distribution of the

FIGURE 1.

EXPERIMENTAL HOUSING



green source was at a maximum near the peak of the rat's visual sensitivity (179, 167); that of the red source was mostly beyond the range of absorption for the rat's retina (167, 179). The rat, a nocturnal animal, has very few cones, exhibits no Purkinje shift either electroretinographically or pupilloscopically (370), and appears to be quite blind to long-wave light. Granit (179) has described a "modulator" element in the light-adapted rat with a peak at 6000 Å, but it is a very sharp peak, and decreases to little or no response by the 6500 Å energy peak of the red light source. Although in general albino eyes, lacking melanin, are more sensitive to the red end of the visible spectrum (106), it seems safe to assume that the rats perceived the red source as, at best, a very dim light.

Light energy levels (89, 258) were measured with an Eppley thermopile and a Kin-Tel galvanometer, model 204A. Window screening, painted flat black, was used as an inexpensive neutral density filter to achieve the desired light levels for each light source. Measured in the center of the second shelf, intensity for red and green lighting was equalized at 100 microwatts/cm^2; for white lighting, at 100 microwatts/cm^2 or at 200 microwatts/cm^2. Other locations in the cabinet had levels from 90 to 120 microwatts/cm^2 for the groups designated "100 microwatts" and from 180 to 220 microwatts for the "200 microwatts" groups. One hundred microwatts/cm^2 is equivalent to about 65 foot-candles of maximum visibility radiation (5560 Ångstrom units).

FIGURE 2

SPECTRAL ENERGY DISTRIBUTION OF WHITE LIGHT SOURCE

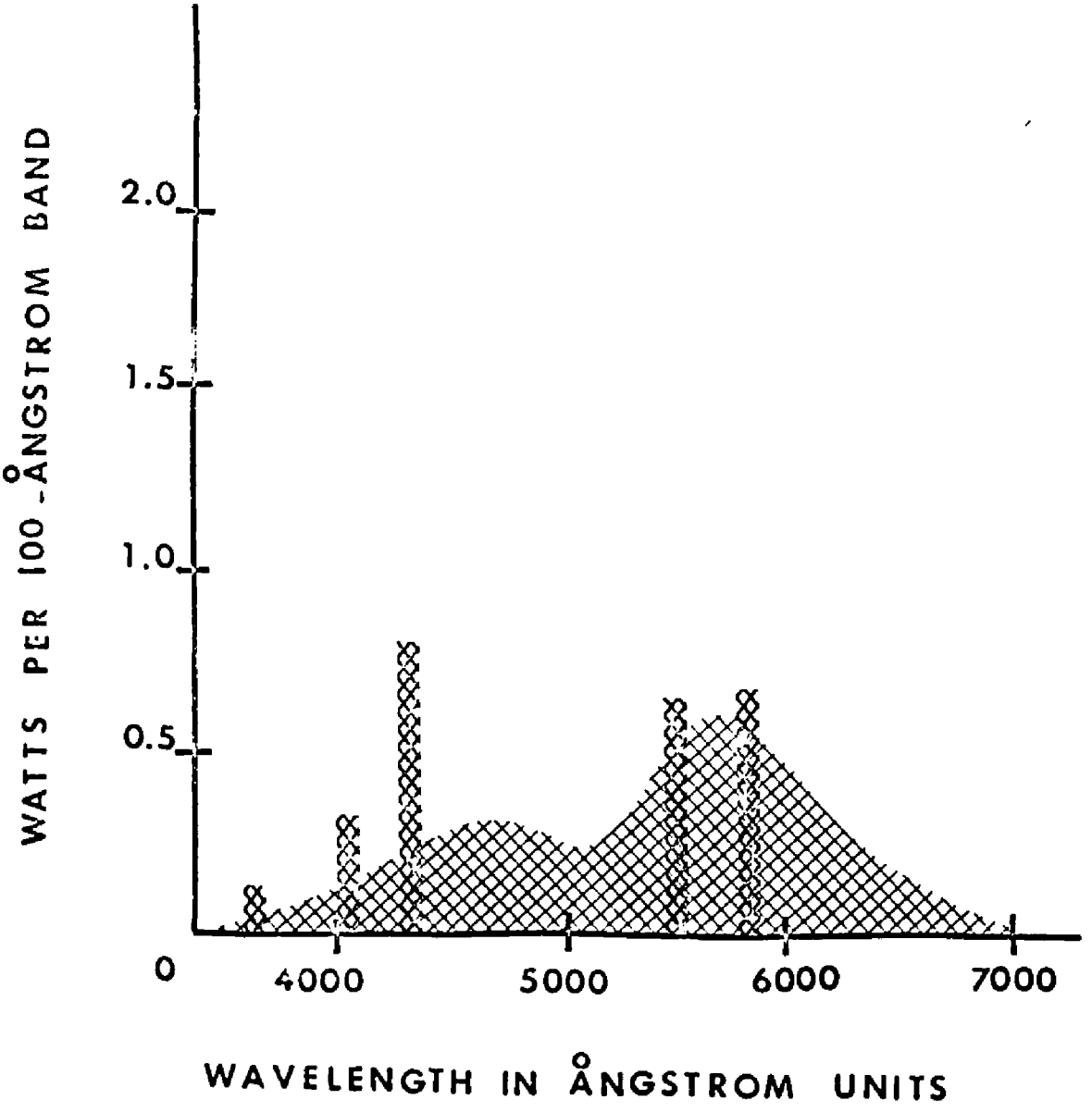


FIGURE 3

SPECTRAL ENERGY DISTRIBUTION OF RED LIGHT SOURCE

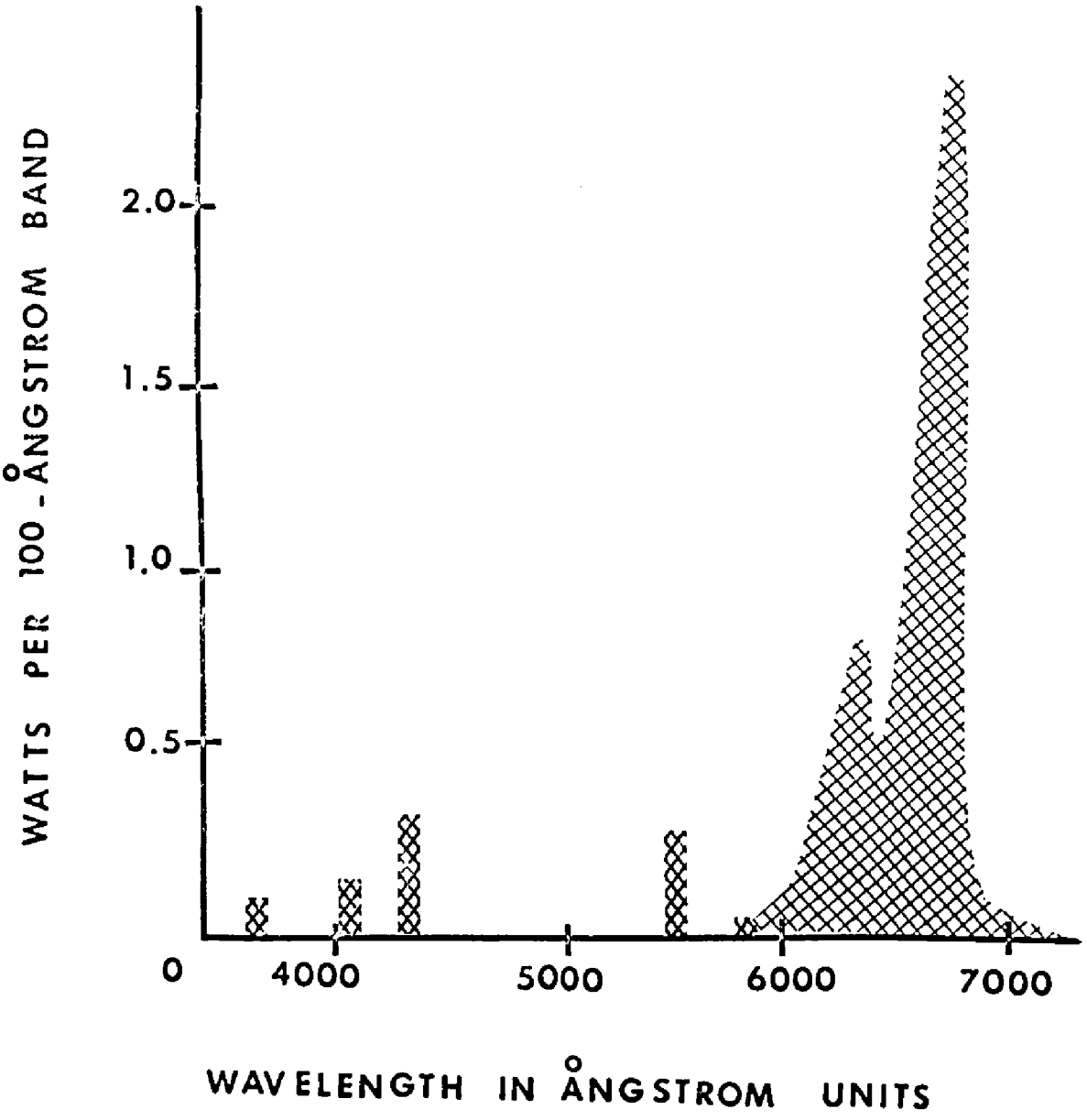
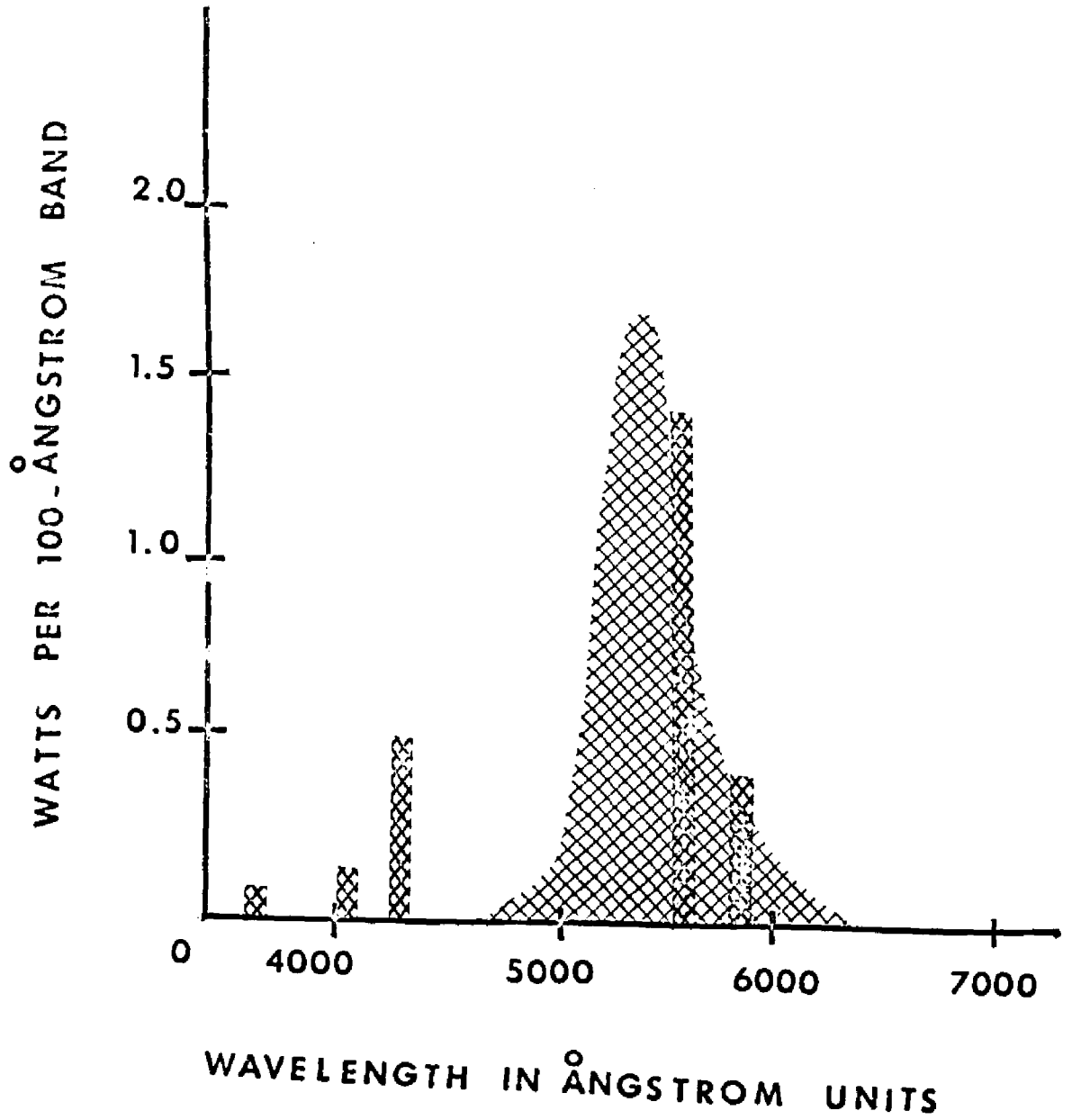


FIGURE 4

SPECTRAL ENERGY DISTRIBUTION OF GREEN LIGHT SOURCE

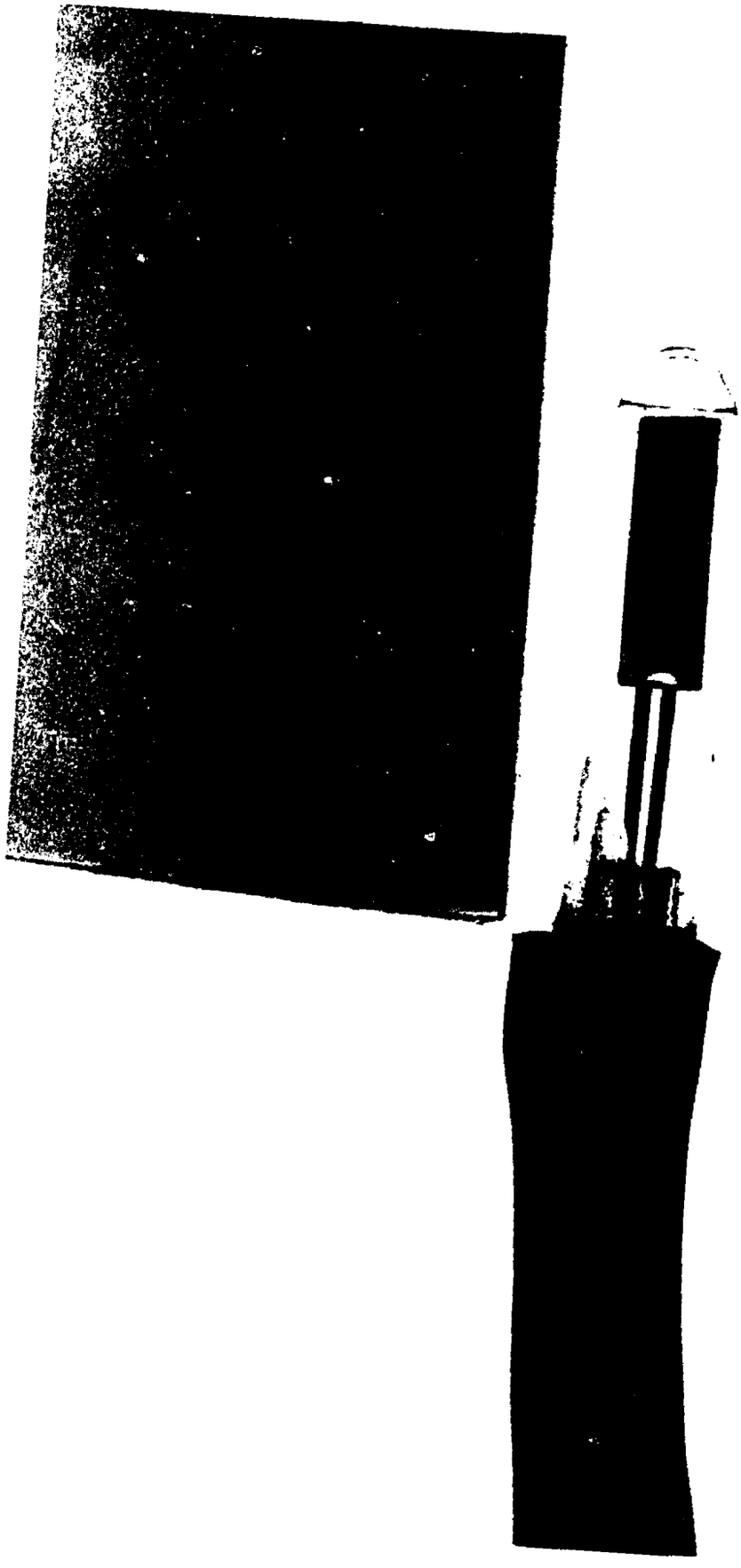


The light penetration measurements were made with a Hoffman (Hoffman Electronics Company - now Centralab - El Monte, California) microminiature photovoltaic detector, model EA7E3, shown in Figure 5. The output current of this device was measured with a Keithley Electrometer, model 621, used on the "fast" setting. The lead wires of the photodetector were coated (Krylon Spray Lacquer) to exclude moisture and enclosed in a single cable for protection and maneuverability. Soldered connections were made to the wires of a longer cable which led to the ammeter input.

The photodetector was inserted into the brain under Nembutal anaesthesia via the parapharyngeal route usually used for hypophysectomy (215). This surgical approach left the top and sides of the rat's head intact, and the small midline cervical incision used for insertion was closed with opaque tape to exclude light and to hold the protruding cable of the photodetector in place. The unconscious rat was then immobilized, dorsal side up, while the penetration readings were taken under each light source. No readings were taken more than five minutes after respiration had ceased; postmortem changes have been shown to affect tissue permeability as early as 30 minutes after death (44, 45). The animal was then sacrificed, if not already expired, and the position of the detector in the brain determined grossly when it was removed. The location of the implant was confirmed by later histological study of the fixed and sectioned brain.

FIGURE 5.

PHOTODETECTOR



Because the response of a silicon solar cell (315) varies enormously over the frequency range of the light sources used, all readings were converted into percentages of incident light transmitted, by the following procedure. For each light source a maximum reading was obtained with the detector taped to the dorsal surface of an anesthetized rat's head or to the palm of a human hand. Either external site had a temperature of approximately 35° C. Then with each implantation of the detector in the brain, a reading was again obtained for each light source, with the detector at the same point in space (with respect to the light source) as previously, but this time inside the rat's head. Temperature during the implantation measurements, monitored rectally, was between 35° and 38° C., depending on the condition of the animal. The direction of the observed temperature difference would tend to distort the percentage transmitted very slightly upward, because the response of the photocell increases with temperature.

SECTION III

RESULTS

It is pertinent to a study of this kind to ask whether, to a nocturnal animal such as the rat, continuous light is not perhaps a stressful, as well as an unnatural, environment. It has been shown that early light exposure increases the acetylcholinesterase activity of the infant rat brain (238). One study reported decreased growth in nursing young rats when housed in constant light (103). Psychologists often use light increments as rewarding or reinforcing stimuli in experiments with rats (49, 259) and at low intensities rats will choose light over darkness. Because the intensities used in these experiments were fairly high, it seemed appropriate to determine whether, apart from their effects on the reproductive system, the light conditions used had any deleterious effects on the animals. Hence the findings on growth and general health are presented first, followed by the effects of light on the various aspects of sexual function.

1. Effect of Light Exposures on Growth and Body Weight

Body weight means for the various lighting conditions and lengths of exposure are shown in Table I; statistical analyses may be found in the Appendix. In general, exposure to continuous light of any frequency or intensity produced body

TABLE 1

BODY WEIGHT MEANS OF FEMALE RATS AFTER EXPOSURE TO VARIOUS LIGHTING CONDITIONS

Lighting	n	Body Weight (G.) ± S.E.M.
200 microwatts/cm^2		
8 weeks		
White continuous light	14	* 221.3 ± 5.6
White 12-12 Dark	15	* 234.9 ± 6.4
30 weeks		
White continuous light	21	* 265.6 5.6
White 12-12 Dark	12	* 287.5 8.2
100 microwatts/cm^2		
4 weeks		
White continuous light	9	182.2 2.1
Red continuous light	10	160.1 5.3
Green continuous light	11	174.1 3.4
White 12-12 Dark	9	185.6 3.1
Control	5	175.6 7.7
8 weeks		
White continuous light	10	254.9 7.1
Red continuous light	16	240.6 5.4
Green continuous light	16	244.2 3.1
White 12-12 Dark	12	255.2 4.5
Control	10	262.6 8.2
15 weeks		
White continuous light	10	294.2 8.1
Red continuous light	21	* 265.1 4.8
Green continuous light	20	* 271.8 5.4
White 12-12 Dark	17	* 261.8 5.6
Control	10	304.6 5.6

weights somewhat lower than those of controls or rats under control lighting or 12-hour photoperiods, but most of the differences are not significant.

At an intensity of 200 microwatts/cm^2, continuous white light tended to reduce body weight as compared to animals exposed to a 12-hour photoperiod of the same intensity of white light. The difference (221.3 vs 234.9 g.) was not significant at 8 weeks of exposure, but by 30 weeks (265.6 vs 287.5 g.) the difference was significant at the .025 level. For this reason the higher intensity was not used for subsequent experiments.

Analyses of variance on the 100 microwatts/cm^2 body weight data shows that treatment differences are significant ($P < .025$) at 4 and at 8 weeks of exposure and highly significant ($P < .005$) at 15 weeks of exposure. However, comparisons among individual treatment means reveals that the various light exposures were not equally effective. For instance, using Duncan's multiple-range test to make comparisons among the 4-week treatment means, the only significant ($P < .05$) differences are between red continuous light (160.1 g.) and white 12-hour photoperiod (185.6 g.), and between red continuous light (160.1 g.) and white continuous light (182.2 g.); red continuous light does not differ significantly from controls (175.6 g.), nor do green or white continuous light differ significantly from controls or from 12-hour photoperiod-exposed rats.

At 8 weeks of exposure, again using Duncan's test, the only significant ($P < .05$) differences are between red continuous light (240.6 g.) and the control lighting (262.6 g.), and between green continuous light (244.2 g.) and the control lighting (262.6 g.). Neither color of continuous light differed significantly from the 12 hour photoperiod exposure; nor did white continuous light (254.9 g.) differ significantly from any other treatment mean at 8 weeks. Thus at this lower intensity white continuous light does not appear to have an adverse effect on body weight at 4 or at 8 weeks of exposure, while red or green continuous light may reduce body weight to a rather variable extent, as compared to control lighting or to a 12-hour photoperiod of white light. It is worth noting that the control and 12-hour photoperiod groups did not differ at 4 or at 8 weeks. Apparently artificial light *per se* does not have any effect on body weight.

At 15 weeks of exposure, red and green continuous light groups have significantly ($P < .05$) lower body weight means (265.1 g. and 271.8 g.,respectively) than control and white continuous light groups (304.6 and 294.2, respectively), yet the red and green groups differ only negligibly from the white 12-hour photoperiod group (261.8 g.). This anomaly is probably due to factors other than the lighting conditions. Among the 15-week groups, all the significant differences in body weight means are between the 1964 groups (RCL, GCL and White 12-12, designated in Table 1 by *), in which SD rats were used, and the 1968 groups (WCL and CON) in which CD rats

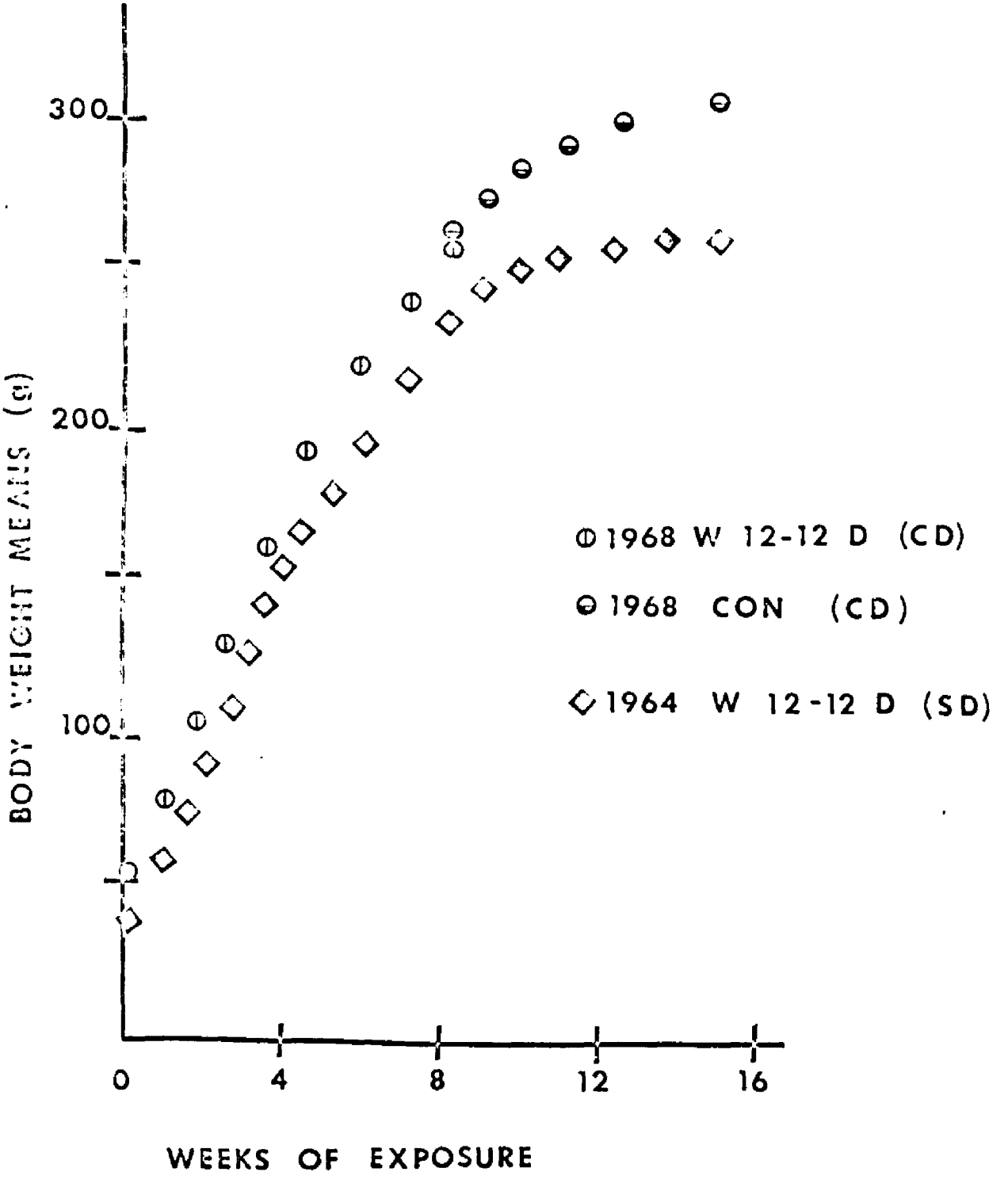
were used. In each case, the control or 12-hour photoperiod groups run concurrently had a body weight mean similar to the continuously lighted group(s). This suggests that the 1964, SD rats tended to be smaller than the 1968 CD rats regardless of lighting exposure.

The SD and CD rats were both Sprague-Dawley strain, but were maintained in different colonies by the supplier. The two colonies apparently differed in vigor. Growth curves for the 12-hour photoperiod and control groups of 1968 (CD) and the 12-hour photoperiod of 1964 (SD) are shown in Figure 6. The two curves compare favorably with those provided by the supplier as representative of his colonies during the two years in question.

It is possible that the differences deriving from the supplier's colonies were augmented by differences in housing the animals during the experiments. During the 1964 (SD) series, due to lack of space, the rats were housed three to a cage up to 8 and 15 weeks of exposure. In the 1968 (CD) series, the rats were reapportioned, two to the same size cage, after 4 weeks of exposure. This hypothesis is especially attractive because the difference in growth in Figure 6 is most pronounced at 8 weeks and after that time. However, three animals per cage still allowed 45 square inches per rat, well above the minimum 29 square inches (143, 381) recommended for rats of 200-300 grams. Furthermore, the 1964 animals did not exhibit any obvious pathological effects of crowding, and the SD growth curve in Figure 6 is well above (25 g. or 10%

FIGURE 6

GROWTH CURVE FOR 1964 AND 1968



at 12 weeks of age) that recorded by the supplier for his SD colony at that time.

These colony and/or housing effects only obscure the light treatment effects on body weight among the 15-week groups, since all the 4 and 8 weeks exposures were done in 1968, using CD rats, while the 200 microwatts/cm^2 series, done in 1964, used only SD rats. If the 15-week white 12-hour photoperiod (1964-SD) group is regarded as another kind of "control", and compared to the red and green continuous light groups run concurrently (1964-SD), neither continuous light exposure had any significant effect on body weight. Similarly, if the 15-week white continuous light group (1968-CD) is compared to the control group run concurrently (1968-CD), continuous light exposure had no significant effect.

In summary, it may be concluded tentatively that red or green continuous light exerts a somewhat depressing influence on body weight at 4 and 8 weeks of exposure, as compared to control lighting or 12-hour photoperiods of white light, but that this effect is probably not significant by 15 weeks of exposure. White continuous light, by contrast, does not appear to reduce body weight for any length of exposure tested, at an intensity of 100 microwatts/cm^2, but does significantly reduce body weight after 30 weeks at 200 microwatts/cm^2.

2. Effect on Light Exposures on General Health, Activity and Behavior

As reported by other workers (206), the activity rhythms of the rats in continuous light underwent some phase

shift and elongation. Usually, nocturnal animals placed in continuous light have a free-running period longer than that shown in diurnal lighting, and higher intensities of light lengthen the period even more (Aschoff's rule) (10). Rats in diurnal lighting are usually active at night, most active on the night preceding estrus (86), and the control and 12-hour photoperiod animals in these experiments conformed to that pattern. They were usually quiescent, until disturbed, at the time of vaginal smearing (9-10 AM), while the rats in continuous light were generally already active at this hour. In fact, many of the continuously-lighted rats seemed to be active at any time during the day when they were observed, while the 12-hour photoperiod animals were often sleeping. It should be noted that the environment of the continuously lighted rats provided some other time clues than lighting; the noise level in the animal room and the daily smearing routine could have acted as Zeitgebers to some extent, and the small diurnal temperature variation might have been sufficient to entrain activity rhythms (66). No entrainment to any of these factors was evident.

Health problems which occurred during the experiments were not limited to the light-exposed groups of rats. A near-epidemic of labyrinthitis occurred during the 1964 series; affected animals were removed as soon as the symptoms (dis-equilibration, circling movements, holding the head at an angle) were noted, and these were excluded from the results.

Only one case of labyrinthitis was found during the 1968 series; it also was omitted from the results.

Several peculiarities of the continuous light exposed animals seem worthy of mention. In general they were hyperactive, less tame, and more easily startled than the control or 12-hour photoperiod groups, which were handled identically. Toward the end of the 30-week exposure at 200 microwatts/cm^2 of white continuous light, some balding of the hind portion of the back was noted. This may have been due to the high estrogen levels in these animals, as observed by Donovan in the ferret (110), or to the repeated mounting behavior observed occasionally in continuously-lighted animals after they had begun to exhibit persistent estrus. This behavior was never observed in control or diurnally-lighted animals, even during their normal estrous periods. Hyperexcitability and increased libido in continuous light were also reported by Jochle (223); it would be interesting to investigate whether these behavioral effects are related to the increased androgen-producing capacity of polycystic ovaries reported by Weisz and Lloyd (371).

3. Effect of Light Exposures on Sexual Maturation

The mean age of vaginal introitus for each lighting condition is shown in Table 2. The 1964-SD rats and the 1968-CD rats are presented separately, since the differences in growth rate, discussed above, may have been accompanied by differences in the rate of sexual maturation.

TABLE 2

MEAN AGE OF VAGINAL INTROITUS OF FEMALE RATS
UNDER VARIOUS LIGHTING CONDITIONS

Lighting	n	Mean Age (Days) ± S.E.M.
200 microwatts/cm^2		
White continuous light	36	37.5 ± .8
White 12-12 Dark	36	39.7 .7
100 microwatts/cm^2 1964		
Red continuous light	21	39.4 1.1
Green continuous light	19	38.1 .4
White 12-12 Dark	15	37.5 .9
100 microwatts/cm^2 1968		
White continuous light	30	37.1 .7
Red continuous light	26	38.4 .8
Green continuous light	27	36.1 .6
White 12-12 Dark	21	39.3 .6
Control	23	38.1 .7

Exposure to 200 microwatts/cm^2 of white continuous light produces a significant ($P < .05$) advancement in the age of vaginal introitus (37.5 vs 39.7 days of age) as compared to rats experiencing a 12-hour photoperiod of the same light.

Among the 100 microwatts/cm^2 1964 groups, the analysis of variance (see Appendix) revealed no significant treatment differences. However, among the 100 microwatts/cm^2 1968 groups, a similar analysis showed treatment differences were significant ($P < .05$). This could be because the 1968 groups were larger.

Comparisons among the various 1968 treatment means, using Duncan's test, shows that white continuous light did have a significant ($P < .05$) accelerating effect on vaginal introitus as compared to a 12-hour photoperiod of the same intensity of white light (37.1 vs 39.3 days of age), but not as compared to control lighting (37.1 vs 38.1 days of age). This may indicate that the accelerating effect of continuous light, reported by other workers, depends rather critically on comparing the continuously-lighted rats to animals which have received a precise photoperiod, rather than the irregular increments of light experienced by the "control" groups in these experiments.

Green continuous light also produced a significant ($P < .05$) decrease in the age of sexual maturity as compared to a 12-hour photoperiod of white light (36.1 vs 39.3 days of age), in the 1968 series, and even as compared to control lighting (36.1 vs 38.1 days of age). Thus green continuous

light appears to be even more effective than white continuous light in accelerating introitus, although this effect is not seen in the 1964 series.

By contrast, red continuous light did not produce any advancement in sexual maturity, in either series, as compared to controls or to 12-hour photoperiod groups. In fact, in the 1968 series, the continuous red light mean age at maturity (38.4 days) was quite close to that of the controls (38.1 days) and differed significantly ($P < .05$) from that of the group exposed to continuous green light (36.1 days).

In summary, white continuous light, at either of the intensity levels used, significantly lowers the age of sexual maturity, as compared to 12-hour photoperiods of the same intensity. Green continuous light can also be very effective in this respect, while red continuous light is not. Other workers (223, 248) have observed considerable individual variation in sensitivity to continuous light stimulation; such differences in "threshold" would probably be most obvious early in photosexual stimulation, and may account for the inconsistency between the 1964 and 1968 series.

4. Effect of Light Exposures on the Vaginal Estrous Cycle

The first few estrous cycles following introitus are usually irregular, regardless of lighting exposure. After this initial erratic period, cycles tended to stabilize in all animals, with a normal length of 4 to 5 days, at least until after 5 weeks of experimental lighting exposure, corresponding

to 8 weeks of age. By this time, all animals had matured, and some had been cycling for two weeks.

At about 6 weeks of exposure, the continuously-lighted groups began to show lengthened periods of vaginal cornification, with, again, wide variation among the animals in each group in the onset and extent of this effect at first. Animals in the same cage, however, tended somewhat to synchronize their cycles, even when these were prolonged. The increase in cornified smears was not due to frequent, or incorrect techniques of, smearing (126), since control and 12 hour-photoperiod groups were also smeared every day, using the same procedure.

The typical prolonged-estrous smear consisted almost entirely of angular, pale-staining cornified epithelial cells, without nuclei. Occasionally nucleated epithelial cells were found, but these were also pale and angular, not small and round as are those of normal proestrous smears. Leucocytes were never present in a smear classified as cornified.

After 8 weeks of exposure there were major differences in the number of cornified smears obtained from controls and 12-hour photoperiod groups on the one hand, and continuously-lighted groups on the other. Because the increase in cornification was a progressive effect, smear data are presented in Table 3 as the average proportion of daily smears which were cornified for each group during a 10-day interval after 6½ and 13½ weeks of exposure; these intervals were the last ten days before autopsy. Each individual rat's proportion of cornified

TABLE 3

AVERAGE PROPORTION OF CORNIFIED SMEARS OBTAINED IN 10-DAY INTERVALS AFTER VARIOUS LIGHTING EXPOSURES

Lighting	n	Average Proportion
200 microwatts/cm^2		
6½ to 8 weeks		
White continuous light	36	.789
White 12-12 Dark	36	.431
28½ to 30 weeks		
White continuous light	21	.962
White 12-12 Dark	12	.540
100 microwatts/cm^2		
6½ to 8 weeks		
White continuous light	20	.815
Red continuous light	16	.637
Green continuous light	16	.625
White 12-12 Dark	12	.525
Control	18	.483
13½ to 15 weeks		
White continuous light	10	.950
Red continuous light	11	.667
Green continuous light	10	.616
White 12-12 Dark	9	.333
Control	10	.400

smears for each 10-day interval was transformed according to the arc-sine percentage transformation and an analysis of variance (see Appendix) was performed on the transformed data. The comparisons among group means were made using Duncan's multiple-range test.

At an intensity of 200 microwatts/cm^2, white continuous light was extremely effective in prolonging vaginal cornification, as compared to a 12-hour photoperiod of the same intensity. The difference was highly significant at 8 and at 30 weeks of exposure, and emphasizes the essentially photoperiodic nature of the effect; a high intensity for only 12 hours a day does not produce a marked increase in vaginal estrus.

The lower intensity of white continuous light is also very effective in prolonging vaginal estrus; the proportion of cornified smears obtained at 8 weeks (.815) and at 15 weeks (.950) is very significant ($P < .01$) greater than that for controls or 12-hour photoperiod rats with the same length of exposure. It may be noted that no exposure used produced the "constant estrus" often referred to in the literature. Even among the rats exposed to white continuous light for 30 weeks, there were still some episodes of leucocytic smears, although these probably did not reflect the occurrence of a normal ovulatory cycle in these animals.

Neither red nor green continuous light produced a significant increase in the proportion of cornified smears at 8 weeks of exposure, although the proportions obtained (.637

and .625) were definitely larger than those from controls or 12-hour photoperiod animals (.483 and .525). By 15 weeks of exposure, both red and green continuous light, with proportions of .667 and .616, respectively, effected a very significant ($P < .01$) increase in vaginal estrus as compared to the 12-hour photoperiod of white light (.333), although only moderately significant ($P < .05$) as compared to control lighting (.400). It may be noted that the increase in significance of the red and green effects between 8 and 15 weeks reflects a smaller proportion of control and 12-hour photoperiod cornified smears more than it does a larger proportion in the continuous light groups. There was, however, less variability among animals in continuous light by 15 weeks of exposure; this fact also tends to increase significance.

To summarize, white continuous light at either intensity is extremely effective in prolonging vaginal cornification, while red and green continuous light are less effective.

5. Effect of Light Exposure on Ovarian Weight and Histology

Ovarian weight means for the various experimental lighting exposures are shown in Table 4. Because of the differences in body weight means discussed previously, the ratio of ovarian weight mean in mg. to 100 g. body weight mean is also presented in the table for each group, to facilitate comparisons. It will be seen that these ratios differ mong comparable treatments to about the same extent that the ovarian weight means do.

TABLE 4

OVARIAN WEIGHT MEANS AFTER EXPOSURE TO VARIOUS LIGHTING CONDITIONS

Lighting	n	Ovarian Weight (mg.) ± S.E.M.		Ratio ov. wt. (mg.) / 100 g. Body wt.
200 microwatts/cm^2				
8 weeks				
White continuous light	15	46.0 ±	3.9	20.6
White 12-12 Dark	15	80.0	4.4	34.0
30 weeks				
White continuous light	21	31.8	1.9	12.0
White 12-12 Dark	12	57.7	3.4	20.0
100 microwatts/cm^2				
4 weeks				
White continuous light	9	58.1	2.6	31.9
Red continuous light	10	48.4	5.6	30.3
Green continuous light	11	54.7	2.5	31.4
White 12-12 Dark	9	56.5	3.3	30.4
Control	5	53.9	4.9	30.6
8 weeks				
White continuous light	10	61.0	5.3	24.0
Red continuous light	16	64.2	4.8	26.6
Green continuous light	16	70.7	6.2	28.9
White 12-12 Dark	12	74.7	3.9	29.3
Control	10	82.0	5.8	31.2
15 weeks				
White continuous light	10	39.3	1.8	13.7
Red continuous light	21	51.6	2.4	19.5
Green continuous light	20	49.6	2.7	18.3
White 12-12 Dark	17	71.3	3.4	27.2
Control	10	83.9	5.6	27.6

The correlation coefficient of ovarian weight with body weight has been calculated by Jackson for large groups of rats (217) to be about .8 after puberty. For the 4-week control lighting group in this experiment, the correlation coefficient was .84. That is, heavier rats of the same age may tend to have heavier ovaries, without experimental lighting exposure. Hence, statistical analysis of treatment differences in ovarian weight must take into account the body weight differences, even though some of the latter were probably not due to treatments.

Analyses of covariance performed on the ovarian weight data (see Appendix) revealed that many of the treatment differences among means were significant even after correction for body weight differences. For all exposures, the covariance analysis increased the precision of comparisons over an ordinary analysis of variance, but it did not change the rank of the means, or render differences among them significant which were not significant by an ordinary analysis of variance. Rather, since continuous light exposure tended to reduce both both weight and ovarian weight, covariance adjustment of the treatment means made the differences among them smaller, but in most cases still significant. Treatment differences among regression coefficients were not significant for any length of exposure. Where the covariance analysis showed significant treatment differences among ovarian weight means, the adjusted means were compared using Wishart's method (376).

White continuous light, at an intensity of 200 microwatts/cm^2, obviously had a very marked effect on ovarian weight, both at 8 and at 30 weeks of exposure, as compared to 12 hours per day of the same intensity. It is noteworthy that even the rats exposed to 12 hours of the 200 microwatts/cm^2 intensity evidenced some diminution in ovarion weight after 30 weeks; this may be correlated with the slightly greater degree of cornification observed in the same animals after 30 weeks. Neither effect can be attributed to old age; the reproductive life of the rat is at least one year. But Everett (128) has reported spontaneous persistent estrus with aging in some strains of rats after 150 days of age (under ordinary day-night lighting), so perhaps such an effect occurred here. Since no control rats were kept for such a long period, a definite conclusion did not seem possible, but the possibility that 12 hours per day of this high intensity might be slightly stimulating to the gonads was one reason that a lower intensity was used for subsequent experiments.

Among the 4 week groups at 100 microwatts/cm^2, no significant differences in ovarian weight were observed, with or without adjustment for the differences in body weight. Also among the 8 week groups, differences among treatment means were not significant, although comparison of the ovarian weight means, or the ratios, for these groups shows that continuous light exposure of all three colors was associated with somewhat lower ovarian weights than was control or 12-hour photoperiod lighting.

By 15 weeks of exposure, differences among treatment means for ovarian weight were highly significant, even after adjustment for body weight. Comparisons among the adjusted treatment means, using Wishart's method, revealed that all the continuous light groups, red, green and white, differed significantly from the 12-hour photoperiod and control groups. In every case the difference is highly ($P < .001$) significant, but as is obvious from the means and the ratios, white light was considerably more effective than red or green in reducing ovarian weight. It is interesting that at none of the autopsy points chosen was an increase in ovarian weight under continuous light found; such an effect is referred to in the literature and was seen in individual rats but other rats in each group apparently had suffered a decrease in weight, so the averages were about the same.

Histological observations were correlated with the observed decreases in ovarian weight. A section of the typical continuous-light-exposed ovary had a Swiss-cheese appearance, with fluid-filled antra of unruptured follicles making up a large part of its area. Oocytes were often degenerating, and fibroblasts were seen invading some follicles. In early stages of light exposure, both cystic and atretic follicles were evident, and usually some corpora lutea. As stimulation by light was prolonged, few corpora lutea persisted, interstitial tissue became reduced, and the ovary appeared to be almost entirely made up of large cystic follicles. Occasionally follicles which appeared to be partly

luteinized were observed; these were also unruptured, but had what appeared to be lutein cells (large, pale, eosinophilic) filling in the antral cavity.

The ovaries of rats exposed to 200 microwatts/cm^2 of white light for 8 weeks had typical light-treated ovaries. Large cystic follicles were present, along with follicles in other stages of normal development, and a few small corpora lutea. By contrast the animals submitted to 12-hour photoperiods of this intensity has essentially normal ovaries, with ripening follicles and numerous, apparently functional, corpora lutea. By 30 weeks at this high intensity, ovaries of rats in continuous light were extremely follicular, typically with a few very large follicles and little else; no functional corpora lutea were seen. At 30 weeks even the 12 hour photoperiod animals at this higher intensity showed some evidence of stimulation; their ovaries were somewhat more follicular than normal, but did contain many corpora lutea.

Among the 4 and 8 week at 100 microwatts/cm^2 exposures, no very striking differences appeared in the ovaries. Continuous white or red light did produce somewhat more follicular ovaries than the 12 hour photoperiod or control lighting; the green-exposed group did not even show this effect. All 4 and 8 week ovaries had functional corpora lutea.

By 15 weeks of exposure at the lower intensity, gross histological differences were evident among the ovaries, according to light exposure. The extreme case was associated with white continuous light; the ovaries of these animals were

very "holey" and follicular, smaller overall in cross section, and had very few corpora lutea, probably none of functional size. By contrast the control or 12-hour photoperiod animals had normal ovaries, made up mostly, in cross section, of corpora lutea, with follicles in various stages of development also evident.

Red or green continuous light at 15 weeks produced an intermediate condition, with many large, often cystic, occasionally atretic, or (rarely) luteinized, and also some corpora lutea. There was marked variation in response among animals, many ovaries being entirely follicular, with no sign of corpora lutea. These often were the ones with the lowest weights at autopsy. The ovaries with the highest autopsy weights, when fixed and sectioned, usually showed well-developed, vascular aid apparently functional corpora lutea. There were not very many ovaries of this latter type, but there were a few in both the red and the green continuous light groups, even at 15 weeks of exposure. This would seem to indicate that ovulation was still occurring in these particular rats; the corpus luteum does not continue to function more than a day or two in the rat unless pregnancy or psuedopregnancy intervene. These same rats were the ones which were still having some diestrous-phase smears after 15 weeks of exposure, which would confirm the histological appearance of functionality in their corpora lutea.

As mentioned several times, the literature reports, and these results confirm, considerable variation among individual rats in sensitivity to continuous light stimulation.

The length of time reported to be required to develop persistent estrus and an anovulatory state under white continuous light varies from 30 days (101) to 100 days (65, 148). This wide range doubtless reflects differences due to age, strain, intensity and perhaps other factors, but a number of authors mention observing individual rats more resistant than others to lighting effects. The relatively few rats which continued to cycle and to ovulate after 15 weeks of exposure to red or green continuous light are probably such resistant individuals. However, since there were no animals presenting this ovarian histology in the 15-week group under white continuous light, and since the ovarian weight mean for white continuous light exposure is considerably lower than that for red or green exposure, it seems safe to conclude that white continuous light is more effective in inducing the anovulatory micropolycystic condition of the ovaries, than is red or green continuous light of equal intensity.

6. Penetration of Light Sources into the Rat Brain

As explained earlier, the electric response of the photodetector used varies quite markedly with the frequency of the incident light, so that direct comparisons among the actual magnitudes of readings taken under the three different light sources would not be valid. Hence the readings were converted into percentages of the incident light from each source, which seems legitimate since the detector's response, measured in microamperes, varies directly with illumination

level, at least over the range of illumination involved.

It was also pointed out earlier that the slight temperature difference between external and internal measurements would tend to distort the percentage transmitted a little upward. The change in the cell's response, as indicated by th manufacturer, is from 92% output at 25° C. to 99% output at 50° C., and the temperature differential was less than 5° C. In any case the amounts of light transmitted are very small: of the order of a few microwatts/cm^2 or about one foot-candle of maximum visibility radiation. These amounts are of course quite large enough to be perceived by the eye.

Table 5 shows the percentages of the various light sources transmitted to different locations in the rat brain. The locations of the implanted photodetector were verified in stained sections, using a stereotaxic atlas (240) for comparison. Obviously the percentages vary a good deal with location, but it should be emphasized that the readings were reproducible; that is, for a given implantation site, the reading obtained for one light source could be verified again under that light source, after other readings under other sources had been taken. Rotation of the light sources in this manner was carried out for two of the four implantations, and for the other two, the white reading was taken at the beginning, and again after the red and green readings. In every case, the second reading varied less than 0.2 microampere from the first. This verification process could not be carried on indefinitely because post-mortem changes have been shown to affect

TABLE 5

PENETRATION OF VARIOUS LIGHT SOURCES INTO THE RAT BRAIN

Location of Detector		Reading (microamps)	% of Outside Reading
Outside head	White	54	
	Red	140	
	Green	110	
Hypothalamus-1	White	.7	1.3
	Red	5.0	3.6
	Green	.5	.5
Hypothalamus-2	White	.9	1.7
	Red	3.2	2.3
	Green	.6	.5
Cerebellum	White	.8	1.5
	Red	4.0	2.9
	Green	1.3	1.2
Mammillary bodies	White	2.0	3.7
	Red	3.4	2.4
	Green	1.0	.9

tissue permeability to light after 30 minutes or so (44, 45).

As Table 5 shows, the percentage transmission of red light through the tissues of a rat's head is consistently greater than that of green light, and in three out of four locations, greater than that of white light. In the exception, the mammillary body implant, the detector came very close to the dorsal surface of the brain.

These measurements then confirm the findings of Benoit on the greater penetration of long-wavelength visible light through tissues. Some theoretical grounds for this finding can be constructed. Light transmitted through any medium is subject to decrements of absorption and of scattering; the sum of these depends in a complex way on the wavelength of the impinging light.

The upper range of absorption of unpigmented protoplasm is 2900-3000 Ångstrom units (96) (the ultraviolet sunburn region), where absorption depends on the aromatic rings of proteins (phenylalanine, tryptophan, tyrosine) and nucleoproteins (purines, pyrimidines). Absorption by living things of longer, visible wavelengths depends on the conjugation of chromophoric configurations (26) in specific molecules such as carotenoids, porphyrins and melanin, which would surely be present in the tissues surrounding the brain. According to Beer's and Lambert's laws, absorption by a particular molecule at a particular wavelength of light is directly proportional to the concentration of that molecule (in solution), and

exponentially related to the thickness of the solution traversed by the light. Thus if all the absorbing compounds present and their absorption bands and concentrations were known, this information might be used to account for absorption in many bands across the visible spectrum. From the cytochrome c Soret band at 4150 Ångstroms to the cytochrome a band at 6050 Ångstroms there would be many bands due to the various porphyrin compounds; beyond this are the flatter absorption curves of carotenoids up to 6500 Ångstroms (218, 292) and of melanin up to 8000 Ångstroms (218, 278). With the exception of melanin, there seem to be more absorbing bands in the short visible wavelengths. This is consistent with the human eye's experience of blood-filled tissues; the red wavelengths are not absorbed but reflected back. An equal proportion of these wavelengths will be transmitted, since both transmittance and reflectance depend on the absorption coefficient.

It should be noted that the absorption of colored compounds in solution, described by Beer's and Lambert's laws, is very different from the situation in the living cell. There is a flattening and broadening of electronic absorption bands when measured in cells. A "sieve effect" (119) is introduced by the fact that the pigment molecules are present intracellularly as small dense particles instead of in solution, and tend to optically shade each other from the incident light. There are probably other distortions of absorption due to intermolecular forces in the heterogeneity of the

living state, reducing the freedom of some molecules to vibrate at certain frequencies. There is also a special type of scattering, the so-called "anomalous dispersion" effect, which attenuates the transmitted light around the absorption bands. This kind of scattering is greatest on the long-wavelength side of the bands and therefore tends to shift their absorption toward the red (243, 244, 245). All of these effects would tend to obscure the pure absorption bands of various compounds present in the tissues.

In any case, the electronic absorptions of specific molecules probably contribute less to the decrement of transmitted light in living systems than do scattering effects. The more general type of scattering occurs even with colorless particles, because of the difference in index of refraction between the particulate matter in the cells and the surrounding aqueous medium. According to Rayleigh's law, which was derived for very small atmospheric particles, the intensity of light scattered by particles of a given size is inversely proportional to the fourth power of the wavelength of the impinging light; short wavelengths are scattered more than long ones. But the scattered intensity for a given wavelength also increases with the sixth power of the radius of the scattering particle (134); large particles scatter more. In fact Rayleigh's relation may not hold for particles whose size is the same order of magnitude as the wavelength of the impinging light; this is the case for visible light (4000-7500 Å) and proteins, which may be hundreds of Ångstroms in in diameter. But in general scattering effects will still

be wavelength-dependent; long wavelengths will be less scattered than short ones, and thus tend to be transmitted to a greater extent.

In a very general way, then, there is theoretical explanation for the greater transmittance of red light through tissues, due both to scattering and to absorption. Especially in the absence of melanin's long-wave absorption (which one would not expect to find in the albino rat), the observed differences in penetration seem somewhat predictable.

SECTION IV

SUMMARY AND CONCLUSIONS

Confirming the findings of other investigators, exposure of female rats to continuous white light was found to accelerate sexual maturity, increase vaginal estrus, and decrease ovarian weight after 8 to 15 weeks of exposure, as compared to 12 hour photoperiods of the same white light. A higher intensity of white continuous light decreased ovarian weight more quickly, but did not seem particularly more effective in advancing the age of introitus or in prolonging vaginal estrus.

Green continuous light at the same strength may accelerate sexual maturity, but red continuous light was not found to have this effect. Both red and green continuous light at the lower intensity produced marked increases in vaginal cornification, but not to the same extent as white continuous light. Both red and green continuous light reduced ovarian weight significantly by 15 weeks of exposure, as compared to 12 hour photoperiods of white light, but neither red nor green was so effective as continuous white light in this capacity.

These findings indicate that the action spectrum for the photosexual effect in the female rat, as in ferrets and birds, is quite different from that for perceptual vision. The green light source, with an energy peak near the peak of visual

sensitivity, should be much more effective for vision than the red; this could be confirmed even by a human observer, to whom the green source seemed much brighter, even though its total energy output was the same in microwatts/cm^2. The greater, or at least earlier, effectiveness of a higher intensity of white light in reducing ovarian weight demonstrates that the lower intensity used for the color comparisons was not a saturating level for the photosexual effect. Red and green light appear to be less effective than white light, and, even more surprising, red and green showed about the same effectiveness.

Several explanations are possible. One is that there is a "magic wavelength" around 6000 Å for the photosexual effect in the rat which was present to a much greater degree in the white source than in the green or red source. This seems unlikely from everything known about the visual process; in general absorption and sensitivity have much broader wavelength distributions. Another possibility is that the wavelengths represented by the mercury resonance lines, in the near-ultraviolet, are the most effective ones; these were present to a greater degree in the white source than in the green or red. This is an interesting possibility because of Ott's work on natural and artificial light's effect on fertility (295). However, if this explanation were correct, the green source should be more effective than the red, because it has a higher energy in this region. A third possible explanation for the effectiveness of white light could be that

it activates two receptors, one in the hypothalamus and one in the retina; while the green source activates only the retinal receptor, and the red source, only the hypothalamic one. It was with this hypothesis in mind that the penetration measurements were undertaken. The somewhat crude results indicate that indeed, more of the red light does penetrate to the brain, and that an encephalic receptor could receive a measurable amount of light in the rat, under the conditions used.

Clearly further investigation is needed to explore this possibility. A possibly definitive experiment could be performed with blinded rats under red light. Another interesting series of experiments could be designed using optic fibers stereotactically implanted in various parts of the brain under various light sources, to determine the brain receptor's action spectrum. If no encephalic receptors can be demonstrated with blinded animals, studies using narrow bands of wavelengths - perhaps the new high-intensity mercury lamps - would be needed to delineate the retinal action spectrum more precisely, and explore the near-ultraviolet range more exactly.

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APPENDIX

ANALYSES OF VARIANCE: BODY WEIGHT

200 microwatts/cm^2; 8 weeks

Source of variation	df	SS	MS	F
Treatment	1	1335	1335	
Error	27	14647	542	2.46 n.s.
Total	28	15982		

200 microwatts/cm^2; 30 weeks

Source of variation	df	SS	MS	F
Treatment	1	3733	3733	
Error	31	22310	719	5.18 *
Total	32	26043		

100 microwatts/cm^2; 4 weeks

Source of variation	df	SS	MS	F
Treatment	4	3702	925	
Error	39	8838	267	3.46 *
Total	43	12540		

100 microwatts/cm^2; 8 weeks

Source of variation	df	SS	MS	F
Treatment	4	5093	1273	
Error	59	22561	382	3.36 *
Total	63	27654		

100 microwatts/cm^2; 15 weeks

Source of variation	df	SS	MS	F
Treatment	4	17780	4445	
Error	73	38097	552	8.5 ***
Total	77	55877		

n.s. Not significant

* significant at .025 or .05 level

** significant at .01 level

*** significant at .005 level

ANALYSES OF VARIANCE: SMEARS

200 microwatts/cm^2; 8 weeks

Source of variation	df	SS	MS	F
Treatment	1	11058	11058	
Error	71	16893	241.8	46 ***
Total	72	27951		

200 microwatts/cm^2; 30 weeks

Source of variation	df	SS	MS	F
Treatment	1	9620	9620	
Error	31	5282	170	56 ***
Total	32	14902		

100 microwatts/cm^2; 8 weeks

Source of variation	df	SS	MS	F
Treatment	4	7842	1960	
Error	77	18896	245	8 ***
Total	81	26738		

100 microwatts/cm^2; 15 weeks

Source of variation	df	SS	MS	F
Treatment	4	21560	5390	
Error	45	13698	304	17 ***
Total	49	35258		

Raw proportions were transformed according to the arc sine percentage transformation; tests among transformed means were made using Duncan's multiple-range test.

n.s. Not significant

* significant at .025 or .05 level

** significant at .01 level

*** significant at .005 level

ANALYSES OF VARIANCE: INTROITUS

200 microwatts/cm^2

Source of variation	df	SS	MS	F
Treatment	1	87	87	
Error	71	1514	21.3	4.14 *
Total	72	1601		

100 microwatts/cm^2: 1964

Source of variation	df	SS	MS	F
Treatment	3	36	12	
Error	52	712	13.7	.8 n.s.
Total	55	748		

100 microwatts/cm^2: 1968

Source of variation	df	SS	MS	F
Treatment	5	153	31	
Error	122	1550	12.7	2.38 *
Total	127	1703		

ANALYSES OF COVARIANCE: OVARIAN WEIGHT

100 microwatts/cm^2; 15 weeks

Source of Variation	df	Sums of products xx	xy	yy	df	Adjusted SS	MS	F
Total	77	55748	5925	27188	76	26559		
Treatment	4	17650	2962	15487				
Error	73	38098	2963	11701	72	11471	159	22***
			Adjusted treatment		4	15088	3772	

n.s. Not significant

* significant at .025 or .05 level

** significant at .01 level

*** significant at .005 level

ANALYSES OF COVARIANCE: OVARIAN WEIGHT

200 microwatts/cm^2; 8 weeks

Source of Variation	df	Sums of products xx	xy	yy	Adjusted df	SS	MS	F
Total	28	15982	7978	15502	27	11619		
Treatment	1	1335	3398	8627				
Error	27	14647	4580	6875	26	5443	209	30***
				Adjusted treatment	1	6276	6276	

200 microwatts/cm^2; 30 weeks

Source of Variation	df	Sums of products xx	xy	yy	Adjusted df	SS	MS	F
Total	32	25983	4013	8219	31	7599		
Treatment	1	3672	4340	5129				
Error	31	22310	-326	3090	30	3085	101	45***
				Adjusted treatment	1	4514	4514	

100 microwatts/cm^2; 4 weeks

Source of variation	df	Sums of products xx	xy	yy	Adjusted df	SS	MS	F
Total	43	12540	5209	5759	42	3595		
Treatment	4	3702	1329	529				
Error	39	8838	3880	5230	38	3526	93	.18 n.s.
				Adjusted treatment	4	69	17	

100 microwatts/cm^2; 8 weeks

Source of variation	df	Sums of products xx	xy	yy	Adjusted df	SS	MS	F
Total	63	25651	13148	25294	62	18553		
Treatment	4	3089	9564	2924				
Error	59	22562	3585	22370	58	21800	376	2.1 n.s.
				Adjusted treatment	4	3247	812	

n.s. Not significant

* significant at .025 or .05 level
** significant at .01 level
*** significant at .005 level