Ocelli and Octopamine and Their Effects on Cabbage Looper Moth Flight Activity

by

Michelle M. Sprint

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of
Master of Science
in
Entomology

APPROVED:

John L. Eaton, Chairman

Donald E. Mullins

Richard G. Saacke

December 6, 1985

Blacksburg, Virginia

M CR
6/27/86

Ocelli and Octopamine and Their Effects on Cabbage Looper Moth Flight Activity

by

Michelle M. Sprint

John L. Eaton, Chairman

Entomology

(ABSTRACT)

The roles of ocelli and octopamine in regulating the onset, intensity, and duration of cabbage looper moth flight activity were examined. This was achieved by studying the flight activity of control, sham, and anocellate moths with and without octopamine treatment. Sham and anocellate moths were produced by cauterization in last-instar larvae, of ocellar primordial cells for anocellate moths, and of non-involved cells for sham moths. Flight activity of moths was monitored by a computerized actograph under normal light (LD) conditions, under advanced-sunset, and under constant dark (DD) conditions to determine the effect of ocelli on flight activity. The role of octopamine was investigated by treating the three groups of moths topically with octopamine dissolved in dimethyl sulfoxide (DMSO) and with DMSO alone, and comparing flight activity before and after treatment.

Results support a combined role for ocelli and the compound eyes in determining flight initiation times, and a role for ocelli in determining flight intensity. These roles were more pronounced in males than in females. It was found that the cauterization operation itself decreases flight activity in sham moths.

No evidence was found that supports the existence of an octopamine effect on flight activity in any of the groups of moths when octopamine is topically applied.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. John L. Eaton, first for accepting as a graduate student in entomology an undergraduate who had never taken an entomology course, and then for making sure she not only mastered the academic subject but developed a deep appreciation for the science. His guidance and good humor were invaluable.

My sincere appreciation to my committee members Dr. Donald E. Mullins and Dr. Richard G. Saacke for their insight, for the use of their laboratories and equipment, and especially for their cooperation and patience while I finished my thesis long-distance.

I also thank my predecessor, for his time spent teaching me about the computer system, and who prepared many of the materials I needed. I thank finally my friend for her energy and support, and my fellow graduate students, too numerous to identify individually, for their companionship.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION

The involvement of "circadian clocks" in the regulation of daily periods of activity in insects and other animals is widely accepted. These endogenous clocks are "set" by environmental stimuli (Saunders, 1976), and activity may be modified to accommodate variations in those stimuli, for example changes in the time of sunset. The mechanisms by which this modulation in response to environmental changes is accomplished are unknown.

Insect ocelli have been suggested to have a role in modulating daily activity periods (Rivault 1976, Goodman 1981, Eaton et al 1983). The ocelli are structurally well-suited for this function and evidence which supports this role is abundant.

Octopamine is one member of a group of compounds known as biogenic amines, some of which have been suggested to be associated with the regulation of periods of insect activity. Octopamine levels in the hemolymph, specifically, have been shown to increase during insect flight (Bailey et al 1983, Davenport and Evans 1984b).

The cabbage looper moth, Trichoplusia ni, shows a distinct rhythm of nocturnal flight activity, which has a circadian component. The ocelli of this insect have been studied extensively (Eaton 1971, 1975, 1976, Dow and Eaton 1976, Eaton and Pappas 1977) and appear to be suited for detection of small changes in light intensity.

The question of whether ocelli and octopamine interact in the modulation of nocturnal flight activity in cabbage looper moths is addressed in this paper. To accomplish this, two objectives were proposed:

1. to examine the role of ocelli in the regulation of flight activity of the cabbage looper moth, and

2. to investigate the relationship between the ocelli and the biogenic amine octopamine in the regulation of activity.

LITERATURE REVIEW

General Ocellus Structure

The ocelli of insects vary widely in form and, possibly, function. They are found in adult insects and the immatures of hemimetabolous insects; at least some members of most orders possess them. In some insects, ocelli appear absent but are actually present either externally, well-hidden, or internally. Dickens and Eaton (1973) discovered that a number of moths and butterflies previously reported as anocellate have a small pair of convex corneal lenses located at the bottom of a round depression in the cuticle and underlaid by cells which may be retinula cells. These structures are extremely small and are usually obscured by scales. In a number of sphingids a nerve-like structure runs between the tiny external ocellus and the internal ocellus reported by Eaton (1971). Internal ocelli are present in other moths (Dickens and Eaton, 1973) and in some ants (Caesar, 1973) as well.

There are usually three ocelli present, arranged in a triangle situated antero-dorsally on the head - on the frons or

vertex, or near the ecdysial line between the two. The most usual form is that of a triangle on the vertex and is found in flies, bees, cicadas, zygopteran dragonflies, stoneflies, grasshoppers, and some praying mantids. When three ocelli are present, the median ocellus shows evidence of a paired origin in that the ocellar nerve is paired, and that the ocellus itself is bilobed in Odonata and a genus of Hymenoptera, Bombus (Chapman, 1982). Sometimes only two ocelli are present as is the case in Trichoplusia ni, the insect used in this study. In other cases, one, four, six, or even eight ocelli may be found, though Bullock and Horridge (1965) point out that the ocelli present in these higher numbers are probably not homologous with the typical ocellus.

The most common ocellus has a single, thickened cuticular lens, though sometimes the cuticle is transparent but not thickened. While the ocellar lens is capable of forming an image, it appears that the image plane lies well behind the receptor cell layer (Homann, 1924; Wolsky, 1930, 1931; Parry, 1947; Cornwell, 1955; Wilson, 1978). Therefore, image-perception is probably not a function of ocelli.

Receptor cells, which range in number from tens to over a thousand, lie in a shallow cup beneath the lens. Though a rhabdomere is formed on each retinula cell and rhabdomeres

from 2 to 7 cells form a rhabdom as in the compound eye, these receptor cells are not arranged in an ordered array as in that structure. Rhabdomeres of irregularly packed cells form a network of baffles (Wilson, 1978), which L. J. Goodman (1981) presumes maximizes the receptor's ability to trap light spread diffusely through the retina. Other specializations for capturing and perceiving light occur as well. In T. ni a rhabdom network across the upper part of the ocellus is formed by 6 to 7 neighboring cells, which are also coupled together by tight junctions (Dow and Eaton, 1976). In various insects, pigment cells may form a ring around the margin of the ocellus, invest the whole ocellus, or may be lacking completely. Some insect ocelli have a movable pigment sheath between the lens and receptor cell layer. Palisade formation, the formation of a clear space around the rhabdom (Chapman, 1982), is reported in the ocelli of S. Gregaria (L. J. Goodman, 1970) and T. ni (Dow and Eaton, 1976). A reflecting tapetum may also be present to increase sensitivity. These arrangements cut down the amount of scattered light which can reduce sensitivity of the system.

The receptor cells are innervated by a small number of large fibers and more numerous smaller fibers. Together they form a discreet peripheral ocellar neuropile area, the ocellar plexus, immediately beneath the receptor cell layer. A second ocellar neuropile area is formed within the tract of

ocellar fibers. This organization converges the output of a large number of photoreceptor cells on a small number of large interneurons. Wilson (1978) points out the suitability of such a system for detection and rapid signalling of small changes in light intensity.

Between insects, differences occur in the way the neural elements of ocellar neuropiles are organized. One difference is the extent to which receptor cell axons interact with each other. Eaton and Pappas (1977) suggest that electrotonic synapses between receptor cell axons in moths could possibly generate synchronized activity in receptor cell axons by reciprocal excitation. Lillywhite (1978) suggests that signal amplification could result if receptor coupling took place by chemical synapses. In the dragonfly and locust, retinal cells and large interneurons are involved in an extensive network of both lateral and feedback synapses (Dowling and Chappell, 1972; L. J. Goodman, 1975; Mobbs. 1978; L. J. Goodman et al, 1979). Dowling and Chappell (1972) suggest that this enables the large interneurons to transmit phasic signals in response to small changes in light intensity. In the locust, the median ocellar neuropile is connected to each lateral ocellar neuropile by two large neurons (C. S. Goodman, 1974; L. J. Goodman et al, 1975). This enables the insect to detect roll, by comparing the

output of the ocelli, for which Cornwell (1955) suggested the Locusta migratoria field of vision was well suited.

Chapman (1982) concludes that ocelli seem structurally adapted for the concentration of light and perception of intensity. I agree with his conclusion but suggest that differences in position of ocelli and in neural pathways and interaction indicate that ocellar function, or at least the relative importance of information perceived and transmitted by ocelli, differs considerably among insects.

Circadian Rhythms and Ocelli

The majority of insects show daily and annual cycles of activity and development (Saunders, 1976). Some of these cycles are in direct response to environmental changes, some are endogenous, and some are a combination of the two. Saunders (1976) defines endogenous cycles with a periodicity close to 24 hours as circadian rhythms. Circadian rhythms have been shown to be entrained by environmental factors such as temperature and light. Activities which follow circadian cycles in insects include locomotion, hormone secretion, and feeding. Diapause, mating, ecdysis, and pupation are some which also have rhythms affected by light and temperature,

and populations rather than individuals are involved in migration, which may have a similar rhythm.

The endogenous nature of circadian and other rhythms is revealed when a light-dark (LD) cycle is changed to a constantly light (LL) cycle or constantly dark (DD) cycle. In this case, the insects' activity follows a "free-running" rhythm which approximates, but is different from, the original rhythm. An insect that, when placed under constant light conditions, shows erratic periods of activity is therefore referred to as "arrhythmic". Phase-shifting the cycles by light or temperature can occur within limits, which vary between insects. Some insect activity, such as the locomotor and ovipositional activity of the stick insect Carausius morosus (Godden, 1973) is entrained entirely by light and has no endogenous, or free-running, capacity.

Saunders (1976) points out be that those circadian rhythms that appear endogenous may in fact be entrained by other factors besides the easily manipulated light and temperature, such as air pressure and periodic fluctuations in gravity. This has since been tested when cultures of Neurospora were studied in a space shuttle flight in which 24-hour time cues were essentially absent (Sulzman, et al, 1984). It was found that the conidiation rhythm of the fungus under space DD

conditions was much the same as that of the fungus grown on Earth under DD.

As previously mentioned, temperature (Page, 1985) and light have both been shown to entrain circadian rhythms. In view of my thesis topic, I will concentrate on light entrainment for the remainder of this review.

The search for the light receptors which entrain activity rhythms has been extensive and results are conflicting. Table 1 summarizes the results of some such studies. Most studies have concentrated on light receptors in the head, though some involved ganglia in the abdomen. Studies of light reception in the head usually involved either destruction of or somehow covering the compound eyes and ocelli, or in some cases covering the entire head and exposing the cerebral ganglia to light. It is important to consider here the implications destroying compound eyes and ocelli and covering them. Vancassel (1968) and Rivault (1976) both point out that when compound eyes or ocelli are covered, by painting or some other method, their receptor cells are sending signals of constant darkness to the brain, whereas when destroyed they are presumably dead and transmitting no information. It is not surprising then that results from experiments using each method are different. Also affecting results of experiments in which eyes or

Table 1. Investigations of location of light receptors entraining activity rhythms

Authors	Insects	Techniques	Effects
Tanaka (1950)	Antheraea pernyi (larva)	cauterized stemmata, caused them to disappear next instar	photoperiodic sensitivity with respect to diapause retained
Cloudsley-Thompson (1953)	Periplaneta americana	painted both eyes and ocelli	caused arrhythmia in locomotor activity
Harker (1955, 1956)	P. americana	destroyed, covered ocelli, cut ocellar nerves	loss of normal locomotor activity
Geispitz (1957)	Dendrolimus pini	covered head or abdomen	showed receptors regulating diapause lie in head
Lees (1960 a & b, 1964)	Megoura viciae	micro-illuminated photoreceptors in head	showed illuminating compound eyes less effective than dorsal midline for sex ratio regulation
Lindauer & Schricker (1963)	Bombus terricola	covered ocelli	found bees with ocelli covered begin to fly later and stop foraging earlier than normal bees
Nowosielski & Patton (1963)	Acheta domestica	blacked compound eyes blacked both compound eyes and ocelli	half the insects showed no distinct re-entrainment prevented re-entrainment
Ball (1965)	P. americana	occluded terminal abdominal ganglion transected ventral nerve cord	caused more locomotor activity in light phase no effect on rhythm
Roberts (1965)	Leucophaea maderae	painted compound eyes	caused free-running locomotor activity rhythm; when paint was removed re-entrainment occurred
	P. americana	removed ocelli	no effect on entrainment
Nishiitsutsuji-Uwo & Pittendrigh (1968)	P. americana	removed ocelli painted entire head and cut optic nerves	no effect on entrainment direct photostimulation of brain caused free-running locomotor activity rhythm
Loher & Chandrashekaran (1970)	Chorthippus curtipennis	both compound eyes and ocelli destroyed or covered, or entire head covered	ovipositional entrainment continued
Ball (1972)	Blaberus craniifer	painted head capsule except small window over protocerebrum	12 of 14 maintained locomotor activity entrainment 3-6 weeks
Dumortier (1972)	Ephippiger spp.	covered entire head or destroyed compound eyes and ocelli	failed to prevent entrainment and re-entrainment of stridulatory activity
Loher (1972)	Teleogryllus commodus	removed compound eyes removed ocelli	caused free-run of stridulatory activity no effect
Godden (1973)	Carausius morosus	ablated compound eyes	failed to interfere with entrainment
Wellington (1974)	B. terricola	covered ocelli covered compound eyes covered bottom 1/2 compound eyes covered ocelli and top 1/2 compound eyes covered top 1/2 compound eyes	at dusk stopped flying and crawling earlier responded slower to changes in polarized light, didn't maintain level flight maintained level flight, didn't fly in cloudy weather, otherwise responded like previous group didn't respond to polarized light on ground, stopped flying earlier behaved normally
Rivault (1976)	P. americana	opaqued ocelli opaqued compound eyes ablated ocelli ablated compound eyes ablated both ocelli and compound eyes	3 of 11 became rhythmic, 1 diurnal, 7 arrhythmic 2 of 10 became rhythmic, 3 diurnal, 5 arrhythmic 5 of 5 rhythmic 5 of 5 rhythmic 4 of 5 rhythmic, 1 arrhythmic
Renner & Heinzeller (1979)	Apis mellifera	opaqued ocelli	returned to feeding site rarely and without regard to trained feeding time
Eaton, et al (1983)	T. ni	occluded ocelli ablated ocelli	delayed flight initiation first day delayed flight initiation first and subsequent days
Fent & Wehner (1985)	Cataglyphis bicolor	painted compound eyes painted ocelli	ants could read compass information from sky to navigate no detectable effect on navigational accuracy

ocelli are covered is the fact that the covering, while at first glance appearing complete and effective, may be incomplete (Renner and Heinzeller, 1979), or that some light may actually be transmitted through or around the covering. Rivault (1976) and Eaton et al (1983), in studies on the locomotor activity of T. ni and P. americana, respectively, got different results from destroying light receptors and simply covering them. Eaton found that when T. ni ocelli were painted over, their flight activity period was delayed only the first day following treatment, while when the ocelli were ablated by cutting them off through the rhabdoms, flight initiation was also delayed on subsequent days. This suggests that ablation was the more reliable technique for preventing light perception.

Considerable variation in activity between individual insects exists even where activity rhythms are well-established. Roberts (1960) used male cockroaches in activity studies because their behavior was "less erratic" than that of females. Leuthold (1966) similarly found that female Leucophaea maderae activity varied with reproductive state. Lipton and Sutherland (1970) found that mature females of P. americana showed no activity rhythm related to light, though virgin females showed a rhythm similar to adult males. Even within males activity varied, with 4% displaying apparently random behavior. Lutz (1932) showed the pattern of A. domestica

locomotor activity varied with individuals and age. Nowosielski and Patton (1963) found that when compound eyes of A. domestica were blacked out, only half the insects showed no distinct reentrainment,, with the other half presumably rhythmic. Ball (1972) painted the head capsules of Blaberus craniifer, leaving a small window over the protocerebrum unpainted. Twelve of the 14 insects studied maintained locomotor activity entrainment, but 2 did not. Rivault (1976) obviously found great variability in her study of P. americana locomotor activity - see Table 1. This inherent variability in insect behavior has certainly contributed to the conflicting mass of evidence for and against a role for ocelli in entraining circadian activity rhythms, and must be considered when drawing conclusions from the literature.

Somewhat aside, it is interesting to note here the similarity between the circadian rhythms of insects and their entrainment and those of other animals. It was previously mentioned that the fungus Neurospora has a distinct circadian rhythm of vegetative growth (Sulzman et al, 1984). Jacklet (1985) points out that in vertebrates, the circadian pacemaker has become localized to specialized tissues, the suprachiasmatic nuclei of the hypothalamus, which is thought to exert its control via hormones and the pineal organ. The pineal itself is a light receptor in some non-mammalian spe-

cies; in mammals information from the eyes is used to entrain circadian rhythms. Ebihara et al (1984) conducted a study of pigeon circadian organization, in which pigeons were blinded or pinealectomized or blinded and pinealectomized. It was found that neither pinealectomy nor blinding abolish circadian rhythms in constant dim light conditions, but that birds which are both blinded and pinealectomized show no circadian rhythms, indicating that both the eyes and the pineal are involved in rhythmicity. In other birds, it was found that pinealectomy alone is sufficient to produce arrhythmicity. It is curious that if the word "ocelli" is substituted for "pineal" in the results of the previous study, they are identical to results from experiments conducted with insects. Perhaps the ocelli are analagous to the pineal in their involvement in the circadian cycle, and perhaps the analogy could be taken a step further, into the expression of circadian rhythmicity and the involvement of such hormones as serotonin and octopamine, some of which are known to be involved with the pineal in mammals, and which are suspected to be involved in activity rhythms in insects.

Octopamine and Activity

The group of compounds known as biogenic amines consists of the catecholamines, dopamine and noradrenaline, the indolalkylamine, 5-hydroxytryptamine (serotonin, 5-HT), and the phenolamine, octopamine. Over the past several years biogenic amines have been found to be ubiquitous in arthropods. Octopamine, specifically, since its discovery in an octopus in 1951 (Erspramer and Boretti), has been found to be present in at least the nervous tissue of all invertebrates studied (Orchard, 1982).

Biogenic amines have been shown to behave as neurotransmitters, neurohormones and, more recently, as neuromodulators. Neuromodulator is a term which has entered the scene only lately - it refers to a neurohormone that either changes the quality of the information being passed through a synapse or changes the spontaneous activity of a receptive neuron or muscle cell (Evans, 1980). In vertebrates, biogenic amines serve as neurotransmitters, neuromodulators, or as true hormones.

Many species of insects have been studied with regard to the location of biogenic amines within the body, and biogenic amines have been found nearly everywhere searched. Fluorescence histochemistry, HPLC, and a sensitive

radioenzymatic technique are all used to study the locations and amounts of biogenic amines in insects. The highest levels of biogenic amines are usually found in the nervous system, with most of this centered in the brain and optic lobes (Evans, 1980). Biogenic amines have also been found in the leg muscle, salivary gland, and fat body of Schistocerca gregaria, and the light organ-containing segments of Photuris versicolis (Evans, 1980). The Apis mellifera nervous system has been extensively surveyed by Mercer et al (1983), who found similar amounts of dopamine and serotonin, and very low levels of noradrenaline, in the cerebral ganglia, with more dopamine present than octopamine. They found catecholamines throughout the cerebral ganglia, with particularly high levels in the central and mushroom bodies. The optic lobes of the honeybee contain the largest amount of octopamine, associated with the neuropile of the medulla. They also contain serotonin and low levels of dopamine.

The first indication that biogenic amines might have a connection with arthropod activity came when Fowler and Goodnight (1966) reported a daily rhythm of biogenic amines in the harvestman, Leiobunum longipes. They found a unimodal cyclic production of 5-HT in brain and intestinal tissues over a 24h period and reported that the maximum biogenic amine content paralleled the peak of activity shown by the harvestman. In 1967, Hinks reported that 5-HT has an effect

on the circadian rhythm of flight activity of Noctua pronuba. When serotoninergic cells in the brain of that Noctuid are ablated, the night flight period is abolished, and the application of 5-HT was found to enhance the duration and amplitude of night flight. This led Hinks to observe that 5-HT appears to play a part in determining the threshold for flight activation in N. pronuba. Fowler et al (1972) also showed a circadian rhythm of serotonin in Drosophila melanogaster, and showed that it is influenced by environmental factors such as season and photoperiod. They showed, too, that the peak production of 5-HT occurs in the middle of scotophase, which again parallels respiratory and locomotor activity patterns as reported by Rensing (1964).

A revealing study was conducted in 1976 by Hoyle and Barker, who showed an identifiable and highly specialized group of neurons to be octopaminergic. These neurons were first reported in the locust in 1969 by Plotnikova and were dubbed Dorsal Unpaired Median (DUM) neurons by Hoyle et al in 1974. They are a median group of cells on the dorsal surface of insect thoracic and abdominal ganglia. Hoyle (1975) subsequently found that when the DUM neurons innervating the extensor tibiae muscle of the locust hindleg (the DUMETi neurons) are stimulated, a slowing of the intrinsic rhythm of contraction and relaxation can be observed and that this effect can be mimicked by application of octopamine.

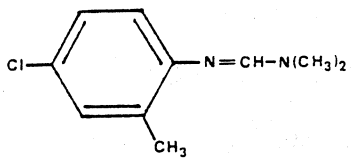
During the last five years, many studies have been conducted in an attempt to discover the extent to which octopamine plays a role in insect activity. Studies in 1980 showed that octopamine content fluctuates over time and as a result of activity. Bodnaryk (1980) conducted one of these studies on brain octopamine levels in the moth Mamestra configurata during metamorphosis. Using a radioenzymatic assay technique, he showed that octopamine increases more than 10X during metamorphosis, and that ultimately the level in the optic lobes was 1.5X that in the rest of the brain. He suggests that the development of an enhanced octopaminergic system during brain ontogeny may be required to modulate input from the compound eyes and that the low concentration of octopamine found in the pupal brain is well-correlated with a low level of sensory input and limited behavioral range of the dormant pupa, which overwinters in the soil at sub-zero temperatures. Also, Goosey and Candy (1980) studied the octopamine content of Schistocerca americana gregaria and its response to flight activity. They showed that octopamine content increases with the advent of flight and then decreases so after 60 minutes of flight it is back to resting levels. They suggest that this indicates a possible physiological role for octopamine in stimulating oxidation of substrates in flight muscle during the early period of flight.

Bailey et al (1983) conducted a similar study with P. americana. They found octopamine concentrations in the hemolymph increase rapidly in response to handling and commencement of flight. They more cautiously support a role for octopamine in "activating or potentiating related physiological processes that facilitate flight." They also support the theory that octopamine mediates a generalized sympathetic-like response to excitation. Davenport and Evans (1984a) found that food deprivation is another stress factor which increases hemolymph octopamine levels in S. gregaria and that this corresponds again to increased speed of movement and total activity compared with unstressed individuals. Those same investigators further studied stress effects on octopamine levels in the hemolymph of S. gregaria and P. americana (1984b) and found an increase in response to a variety of stimuli - up to 10X increase in locusts and 3X in cockroaches. They also found considerable variation, almost 2X, in basal octopamine levels. They confirmed that in both insects studied the highest hemolymph concentration of octopamine coincides with the peak of locomotor activity. David and Verron (1982) found that the heads and bodies of hyperactive ants had a significantly higher octopamine content than heads and bodies of hypoactive ants.

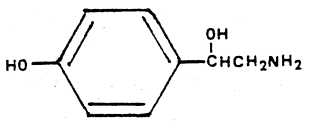
When octopamine is applied topically or injected, it affects a variety of behaviors. It stimulates feeding behavior and

induces hyperphagia in blowflies, as does its agonist, chlordimeform (see Figure 1) (Long and Murdock, 1983). It was also shown in that study that the octopaminergic receptor antagonist yohimbine prevents the hyperphagia induced by octopamine and chlordimeform.

Topically applied octopamine increases sensitivity of the oriental fruit moth to an olfactory pheromone signal, which response is again mimicked by chlordimeform and blocked by yohimbine (Linn and Roelofs, 1984). In this study the differences observed were not statistically significant, but were associated with other deviant behavior which made them biologically significant. Octopamine released iontophoretically into neuropile regions of the locust metathoracic ganglion induced the following responses: repetitive flex-extend-flex movements of tibiae, stepping movements of flexor muscle, and flight motor activity. When released into the 6th abdominal ganglion, it caused suppression of ovipositional digging (Sombati and Hoyle, 1984a). Also in the metathoracic ganglion, octopamine dishabituated EPSPs which were habituated from a variety of inputs onto motor neurons (Sombati and Hoyle, 1984b); when larger amounts of octopamine were applied, the EPSP became larger and in many cases action potentials were initiated.



CHLORDIMEFORM



OCTOPAMINE

Figure 1. Structures of the insecticide chlordimeform and the biogenic amine octopamine.

Jahagirdar et al (1984) showed that injection of octopamine into adult male P. americana causes elevated trehalase activity in both hemolymph and muscle. Related to this are findings by Orchard et al (1982) and Pimley (1984). Orchard found that octopamine is capable of stimulating isolated fat body to release lipid, and Pimley found that octopamine will stimulate lipid mobilization and proline synthesis while inhibiting lipid systhesis from leucine in the tsetse fly. Together, these observations lend support to the idea that octopamine may be to insects what adrenaline-noradrenaline is to vertebrates in preparation for the fight-or-flight (the latter literally, for most insects) response. Orchard and Lange (1984) further investigated the relationship between locust fat body and octopamine and found that flight in the locust induces two phases of cyclic AMP elevation - one during the first 10 minutes of flight and a second between 20-30 minutes into the flight. They concluded from their observations that the previously reported increased titre of octopamine and subsequent fall is responsible for the first increase in cAMP level, which stimulates the initial mobilization of lipid seen with 10 minutes of flight.

Goosey and Candy (1982) investigated the release and removal of octopamine in S. americana gregaria and found the octopamine content of the nerves of the dorsal longitudinal flight muscles and of dorso-ventral nerve-muscle tissues is

lower in locusts flown for 10 minutes than in unflown controls, and that endogenous octopamine is released from working thoracic muscle preparations perfused in the presence of yohimbine. They proposed, then, that the release of octopamine from thoracic nerves can account for much of the increase in hemolymph octopamine that occurs during the first few minutes of flight. They also found that Malpighian tubules are effective at removing octopamine *in vitro*.

The literature represented in this review raises an interesting question regarding the relationship, if any exists, between insect ocelli and the biogenic amine octopamine. It has been shown that the ocelli play a role in light entrainment of activity rhythms, and octopamine appears to be involved in modulating those same activities. The first objective of my research, then, is to confirm the role of ocelli in the flight activity of the cabbage looper moth, *Trichoplusia ni*, and the second to investigate the relationship between that ocellar role and octopamine.

MATERIALS AND METHODS

GENERAL

The cabbage looper moth, Trichoplusia ni, Hubner (Lepidoptera: Noctuidae) was the subject of this research. It has been reared at Virginia Tech on a modified wheat germ diet (Henneberry and Kishaba, 1966) for 10 years. Adults were fed 10% sucrose in water. Field-collected pupae were periodically introduced into the colony to increase genetic diversity.

Three groups of 40 late-5th-instar larvae were carbon dioxide anesthetized for all experiments. The larvae of the control group were allowed to recover after anesthetization. The second and third groups were both cauterized while anesthetized. The cauterization technique was developed by Eaton (1983), and involved destroying the ocellar primordial cells with a small hot electrical wire so no ocelli would develop during metamorphosis. Sham moths were produced by cauterization farther forward on their head capsules (see Figure 2). Treated larvae were returned to an environmental chamber and allowed to complete development.

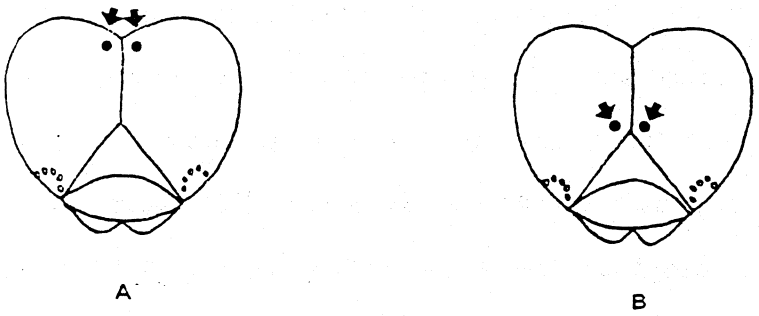


Figure 2. Cauterization points.
Frontal view of larval head capsule.
Dots with arrows indicate points of cauterization for production of anocellate (A) and sham-treated (B) moths.

Pupae were separated into sex/treatment groups and held in the environmental chamber under a 14L:10D photoperiod. After emergence, moths with ages plus/minus 24h were examined for presence or absence of ocelli and placed individually in one of 32 flight cages within a computerized actograph, which was also housed in the environmental chamber. Flight cages were cylindrical cardboard containers, 13 cm diameter x 16 cm height, with a wire mesh top, on which vials with sponges soaked in sucrose solution were placed for moths to feed ad libitum (see Figure 3). Moths were maintained under a light regime that consisted of a 1 h simulated sunset, 9 h dark, 1 h simulated sunrise, and 13 h light. Two 1.25 cm x 2.5 cm rectangular windows were cut in the sides of each flight cage, 2 cm from the top, opposite each other across the diameter of the cage. A light-emitting diode on one side of the cage produced an infra-red light beam which entered through one of the windows and was detected through the window across the cage by a photo-transistor. When a moth broke the light beam it was detected and recorded by the computer as a flight (Eaton, 1985).

The actograph had temperature, humidity, and light intensity sensors and interfaced to a DEC MINC-11 computer which recorded data (see Figure 4). This could be accessed via a DEC VT105 terminal. The University IBM 370 computer could

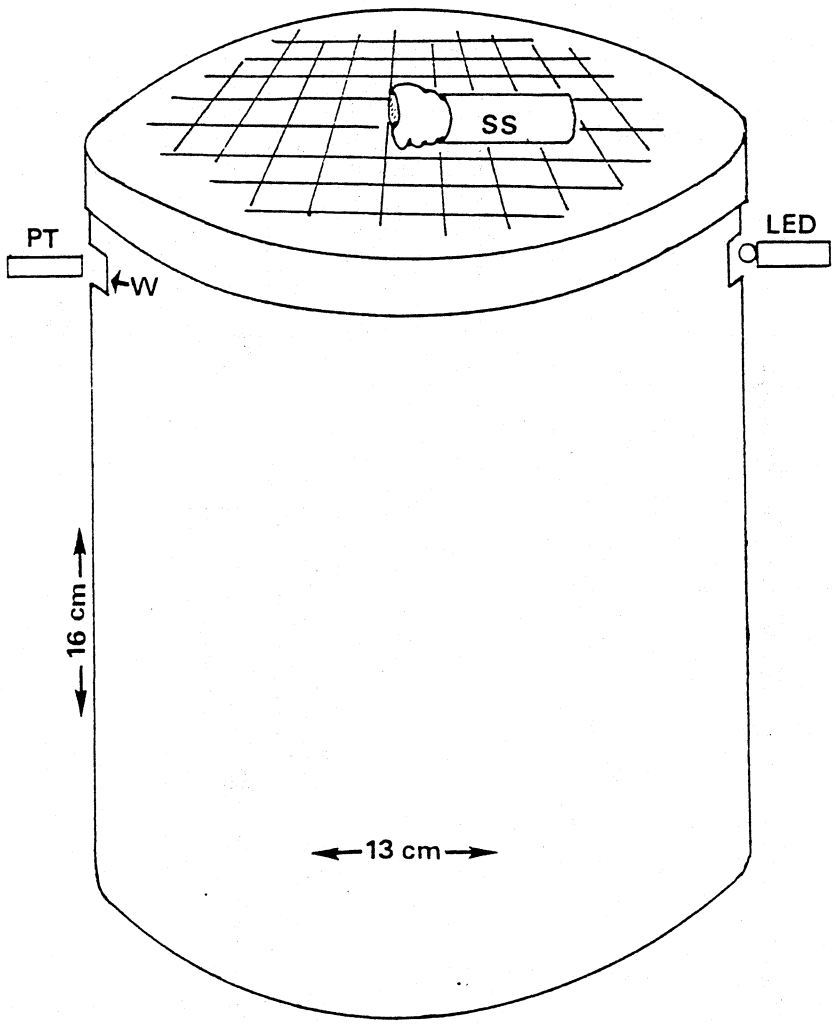


Figure 3. Diagram of a flight cage.
PT=Photo-transistor
W=Window
SS=Sucrose solution
LED=Light-emitting diode.

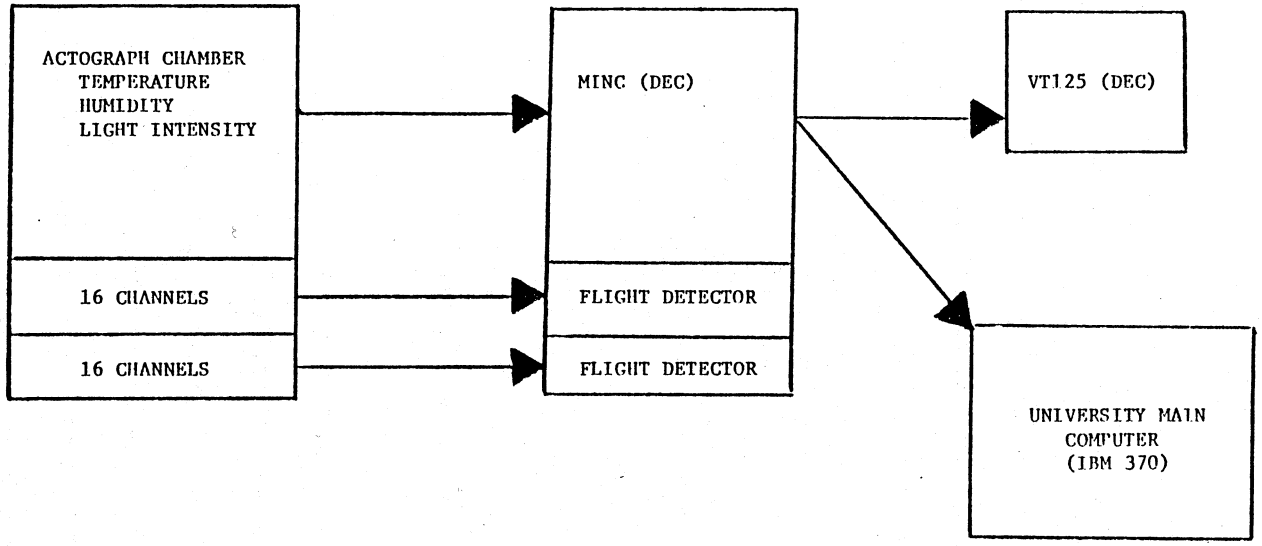


Figure 4. Schematic diagram of actograph and associations.

also be accessed for data analysis. By this system onset, intensity, and duration of moths' flight could be measured.

The environmental chamber was maintained at 25°C with simulated sunset starting at 4:00 pm EST and simulated sunrise beginning at 1:00 am (Byers and Unkrich, 1983). Anocellate moths were autopsied after the first three experiments to verify that no ocellar remnants were present. This practice was discontinued when it was concluded that the cauterization was complete. Flight data from moths that died during the experiment, as well as data from moths that, for any reason, remained flightless for two consecutive days was discarded.

I. OCELLI AND FLIGHT ACTIVITY

To pursue the first objective, examination of the role of ocelli in the regulation of flight activity of the cabbage looper moth, experiments were conducted in which flight activity of ocellate and anocellate moths under normal light conditions was recorded and analyzed. Then, in a second set of experiments, photoperiod was manipulated so sunset occurred earlier ("phase-advance"), and flight activity was again recorded and analyzed. Finally, in a third set of experiments, moths were maintained in constant darkness ("free-run"), flight activity was recorded and analyzed, then

a normal photoperiod was reinstated and again flight activity was recorded and analyzed.

To pursue the second objective, investigation of the relationship between the ocelli and the biogenic amine octopamine in the regulation of flight activity, ocellate and anocellate female moths were untreated, treated with dimethyl sulfoxide (DMSO), and treated with 10% octopamine in DMSO. They were maintained under a normal photoperiod and their flight activity recorded and analyzed.

A. Flight onset, intensity, duration under normal lighting

Experiments with each set of moths in the actograph lasted between 1 and 5 days, and were repeated to give a total of 474 moth/days, which broke down into treatment groups as follows:

91 control male moth/days

87 control female moth/days

72 sham male moth/days

95 sham female moth/days

53 anocellate male moth/days

76 anocellate female moth/days

474 total moth/days.

A moth/day as it is used here is the period one moth is monitored; it extends from 3:30 pm one day to 4:30 am the next.

Three groups of analyses were performed on the data collected from these experiments. The first two involved flight intensity; the first examined total numbers of flights per night per treatment group, the second divided the monitored period into three intervals and treated them separately. The third examined times of flight onset, cessation, and duration.

In all cases, a nonparametric analysis was performed on the data. The test used was the Kruskal-Wallis analysis of variance by rank (Kruskal, 1952).

B. Phase-advance

Only males were used in this experiment: 10 control, 10 sham, and 7 anocellate. They were placed in the actograph and allowed to habituate for one night before their activity was monitored. Then after activity was monitored one night under the normal photoperiod, the time of sunset was advanced 2h, so instead of commencing at 4:00 pm it began at 2:00 pm. Activity was monitored for 2 days under the new light regime, then the time of sunset was returned to normal, and activity monitored again for one night.

C. Free-run

Males only were used in this study: 10 control, 10 sham, and 9 anocellate. Here again, moths were placed in flight cages and allowed to habituate before monitoring began the second night. Then after sunset the third night, the light control was disconnected to establish constant darkness (DD). During DD care was taken when replenishing sugar water vials that no flashes of light would reach the flight cages and disturb the moths. Activity was monitored 2 days under constant darkness, then the normal light regime was re-established and activity monitored for an additional 2 days.

II. OCTOPAMINE AND FLIGHT ACTIVITY

Female moths were used in this set of experiments because previous experiments showed them to be more homogeneous in their flight initiation at sunset.

Octopamine was obtained from Sigma Chemical Co., St. Louis, MO, as the hydrochloride. Dimethyl sulfoxide (DMSO) was obtained from Fisher Scientific Co., Fair Lawn, NJ, and was used as the solvent for octopamine. Linn and Roelofs (1984) showed DMSO to carry octopamine across the cuticle.

Female moths were treated with DMSO alone, with 10% octopamine in DMSO, or were left untreated in the following numbers:

9 control - no treatment

10 control - DMSO

12 control - 10% octopamine

9 sham - no treatment

9 sham - DMSO

11 sham - 10% octopamine

9 anocellate - no treatment

9 anocellate - DMSO

12 anocellate - 10% octopamine

90 total moths.

Applications of solutions were made topically to the dorsal thorax, which had previously been brushed free of scales. A 1 µl application for each was made with a Drummond Scientific Co. "Microcaps" disposable micropipette. The octopamine solution was prepared fresh each time, and a new micropipette was used for each set of applications. Most moths were not disturbed by the application procedure.

The moths were allowed to habituate in the actograph, then monitored for one night. At sunset the next night applications were made and activity was monitored. Data were subjected to analyses similar to those described in the "Flight Onset, Intensity, and Duration" section of this thesis.

RESULTS AND DISCUSSION

I. OCELLI AND FLIGHT ACTIVITY

A. Flight onset, intensity, and duration under normal lighting

1. Total flights

In these experiments, sham, control, and anocellate moths of both sexes were placed in the actograph and their activity monitored for 1-5 days. Then the data was analyzed, in the ways described, by the Kruskal-Wallis test. For this particular analysis mean numbers of total flights during a moth/day were compared. Figure 5 shows mean flight values for each experimental group. The analysis revealed that sham and control males flew significantly ($p \leq 0.05$) more than the other groups, but not differently from each other. Significant ($p \leq 0.05$) differences also appeared among female groups, with controls flying significantly more than both shams and anocellates. This is the first evidence of a phenomenon that becomes increasingly obvious throughout analysis - that in many cases the cauterization operation itself

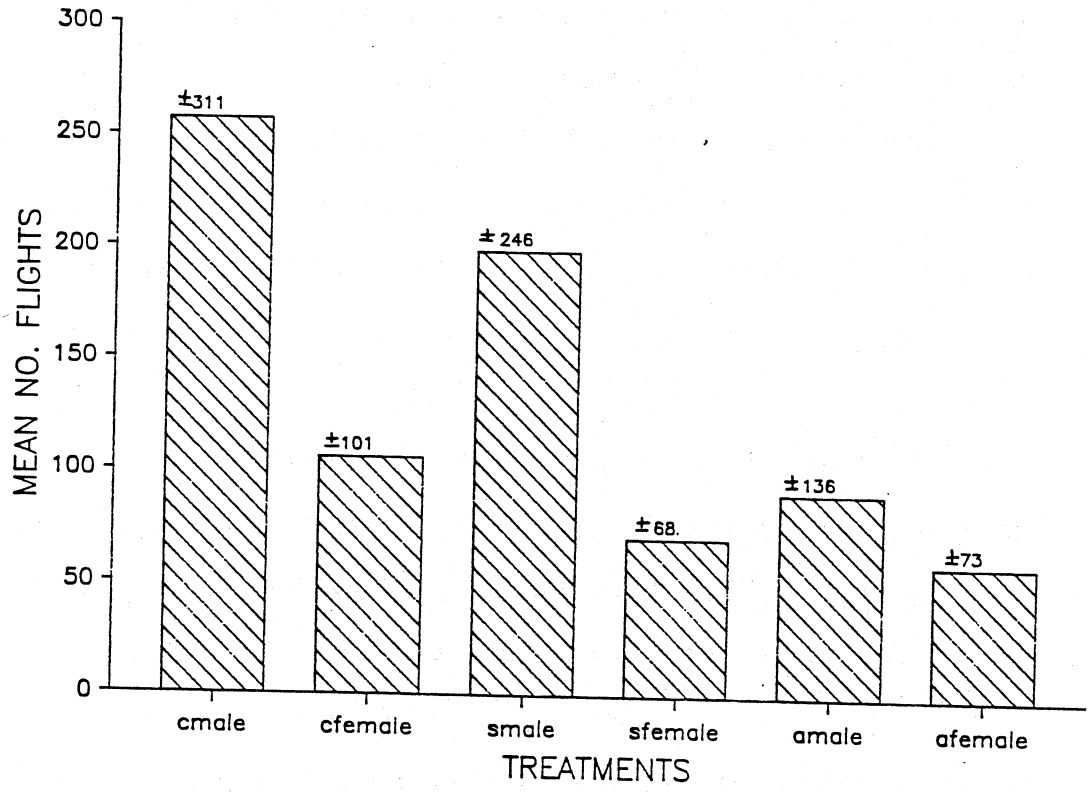


Figure 5. Mean Number of Total Flights.
Numbers on top of the bars are standard deviations.

cmale = control males, n=91	a
cfemale = control females, n=87	b
smale = sham males, n=72	a
sfemale = sham females, n=95	c
amale = anocellate males, n=53	c
afemale = anocellate females, n=76	c

Groups followed by the same letter are not significantly different (Kruskal-Wallis Analysis of Variance by Rank, $p \leq 0.05$).

has an effect on cabbage looper moth flight activity. Also evident for the first, but not the last, time is a pattern which appears in total numbers of flights. As can be seen in Figure 5, numbers of flights decrease from control males (260) to shams (195) to anocellates (90), with females behaving the same way (105, 70, 60, respectively) to a lesser degree.

The standard deviations here are all large compared with the values themselves (Figure 5); the inherent problems with individual variability in studies of this type are evident, nevertheless results point to some trends.

The lack of significant difference between sham and control males is important as it implies an absence of significant effect due to the cauterization operation itself. This seems contradictory when among females it appears the operation does have an effect. I believe the significance of the "operation effect" is more a function of degree than of sex. That is, females fly less than males so the effect is relatively greater among them; the smaller flight number coupled with an inherently great variability and an operation effect could obscure whatever differences might otherwise exist. The fact that the females reflect the pattern of the males in their mean number of total flights supports this view.

The results of this analysis indicate that ocelli do indeed have an effect on flight activity; male cabbage looper moths with ocelli fly significantly more than those without.

2. Number of flights for intervals

It was found in preliminary studies (Eaton, 1985) that consistent peaks of flight activity occur during the night. To further examine the differences in numbers of total flights, a study of flight activity in three nightly intervals was performed. The first interval consisted of the period from 1/2 h before to 4-1/2 h after sunset (3:30-8:30 pm). It was intended to contain the first of the flight peaks seen in preliminary studies. The second interval began when the first ended (8:30 pm) and spanned the next four hours, at which time the third interval began. The third interval, then, comprised the period between 12:30 am and 4:30 am and included sunrise. It was again intended to include an activity peak. The middle interval appeared less consistent with respect to flight activity in the preliminary studies, sometimes containing what seemed to be peaks, sometimes not. Mean numbers of flights for each interval were analyzed. The results of this study are presented in Figures 6-8.

The mean flights for the first interval are represented in Figure 6. In this interval control females flew

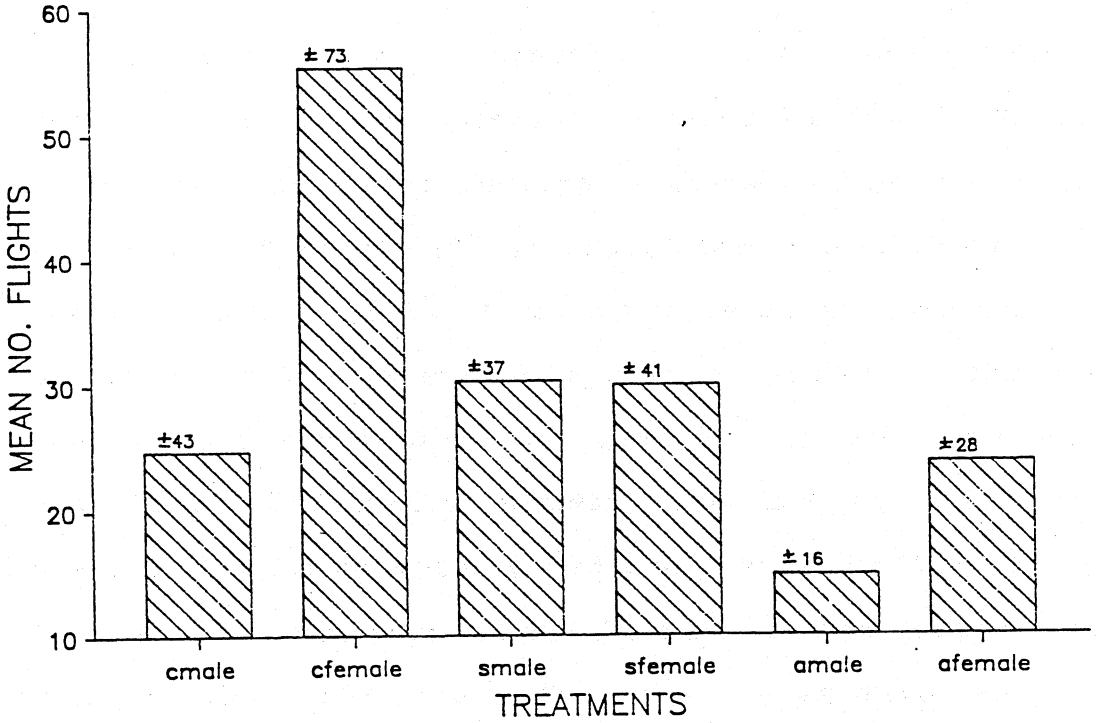


Figure 6. Mean Number of Flights During the First Interval. Numbers on top of the bars are standard deviations.

cmale = control males, n=91	bc
cfemale = control females, n=87	a
smale = sham males, n=72	b
sfemale = sham females, n=95	bc
amale = anocellate males, n=53	c
afemale = anocellate females, n=76	c

Groups followed by the same letter are not significantly different (Kruskal-Wallis Analysis of Variance by Rank, $p \leq 0.05$).

significantly more than any other group (56 flights, $p \leq 0.05$), including sham females (29 flights), indicating again the possible presence of an operation effect. An operation effect is possibly also present in males in this interval, but is not consistent with females; sham males flew significantly more than either control or anocellate males. Note that the mean numbers of flights in this interval have low values again (under 100) when the operation effect is manifested. The results are meaningful, however, if one considers the significant difference between sham and anocellate males. Since both sets of moths would presumably exhibit the operation effect, presence of ocelli must be the factor contributing to the difference. So again, males with ocelli flew more than males without.

The second interval shows quite a change from the first and the pattern described previously under total flights again appears (Figure 7). During this interval males flew more than their female counterparts (170 vs. 30, 110 vs. 25, 55 vs. 20 flights, for controls, shams, and anocellates respectively), significantly so ($p \leq 0.05$) for control and sham groups. The mean number of flights is much higher for males in the second interval than in the first; this is when male flight peaks. The anocellate males' peak is only one-third the level of the control males'. This difference is statistically significant, as is the difference between the sham

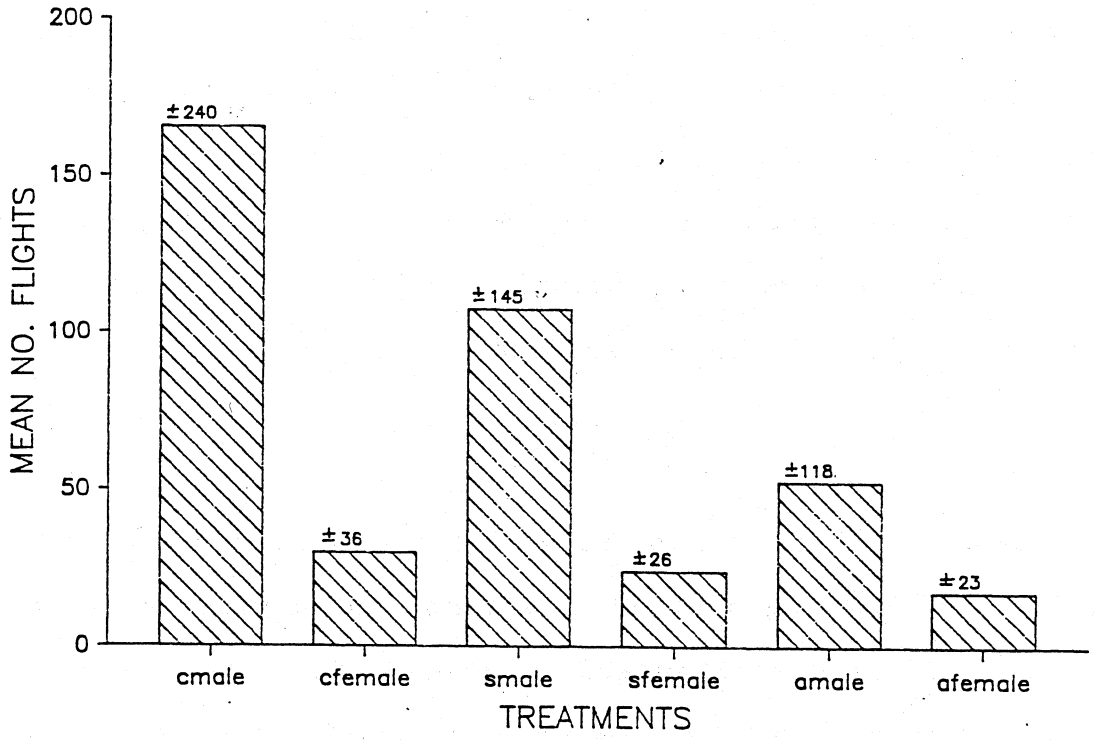


Figure 7. Mean Number of Flights During the Second Interval. Numbers on top of the bars are standard deviations.

cmale = control males, n=91	a
cfemale = control females, n=87	b
smale = sham males, n=72	a
sfemale = sham females, n=95	b
amale = anocellate males, n=53	c
afemale = anocellate females, n=76	c

Groups followed by the same letter are not significantly different (Kruskal-Wallis Analysis of Variance by Rank, $p \leq 0.05$).

males and the anocellate males. Controls and shams are not significantly different. Here is good evidence for an ocellar effect on flight activity. Though it appears from Figure 7 that sham males (110 flights) flew less than control males (170), with the higher flight numbers the significance of the operation effect disappears. No significant differences were found among females, which had very low flight numbers.

The third interval (Figure 8) shows control males flying significantly more than the other groups, at a lower level than in the first interval. During this interval the familiar pattern is shown among males, but the operation effect - sham males weren't significantly different from anocellates, while they were from controls - makes it impossible for anything definite to be said regarding an ocellar effect. There were again no significant differences among females.

It would appear from the number of times control and sham males are shown to fly significantly more than anocellate males that ocelli do indeed have a pronounced effect on flight intensity, presence of ocelli leading to a greater intensity of flight activity. In the females the presence of an operation effect combined with a large standard deviation makes it impossible to establish a clear relationship among them regarding flight activity.

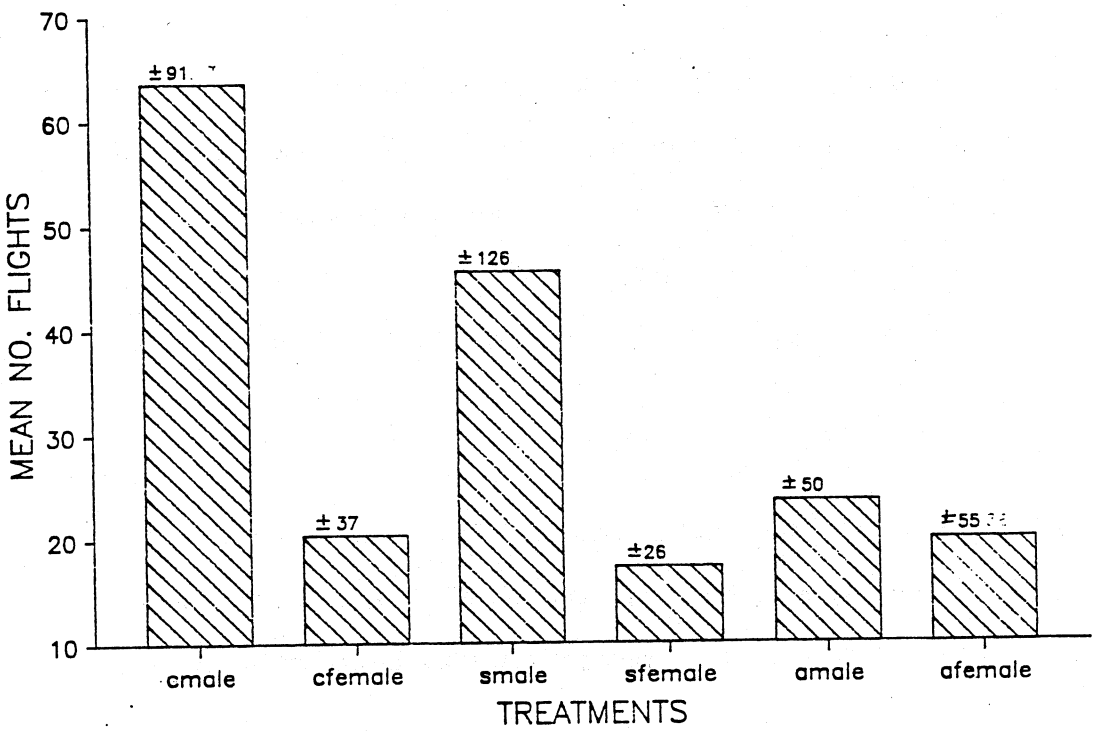


Figure 8. Mean Number of Flights During the Third Interval. Numbers on top of the bars are standard deviations.

cmale = control males, n=91	a
cfemale = control females, n=87	b
smale = sham males, n=72	b
sfemale = sham females, n=95	b
amale = anocellate males, n=53	b
afemale = anocellate females, n=76	b

Groups followed by the same letter are not significantly different (Kruskal-Wallis Analysis of Variance by Rank, $p \leq 0.05$).

3. Flight Initiation, Cessation, and Duration

The third analysis group for the first part of my objectives comprised flight initiation, cessation, and duration. In this analysis, the times of three events were obtained:

- The number of hours after observation began (i.e., 1/2 h before sunset started) the moths started to fly. Flight initiation was chosen to occur when the moth's number of flights had reached 5. Five flights were chosen as the threshold for the beginning of flight activity for the night.

- The number of hours after observation began the moths stopped flying. This was an obvious point at which the flights stopped accumulating.

- The difference between the two figures above, that is, the duration of the flying time.

a. Flight initiation: Figure 9 shows the mean times of flight initiation for each experimental group. It was found

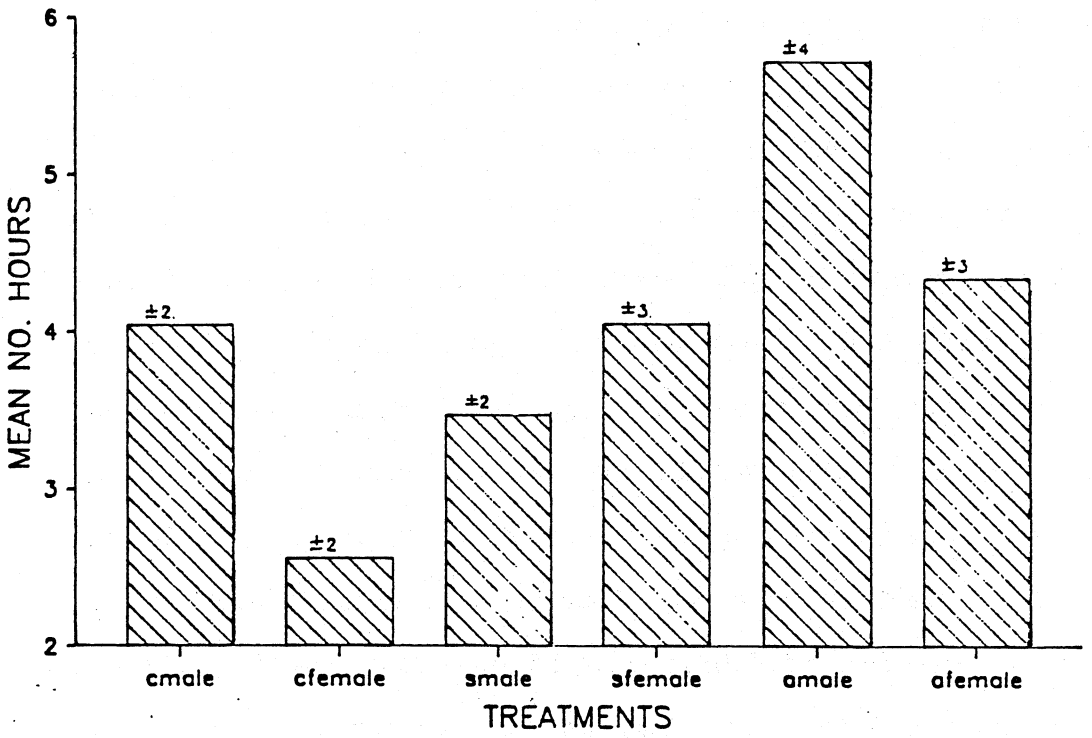


Figure 9. Mean Times of Flight Initiation.
Y-axis represents the mean number of hours after observation began (1/2 h before start of sunset). Numbers on top of the bars are standard deviations.

cmale = control males, n=91	bc
cfemale = control females, n=87	a
smale = sham males, n=72	b
sfemale = sham females, n=95	b
amale = anocellate males, n=53	c
afemale = anocellate females, n=76	bc

Groups followed by the same letter are not significantly different (Kruskal-Wallis Analysis of Variance by Rank, $p \leq 0.05$).

that control females began flying significantly ($p \leq 0.05$) earlier than control males and sham and anocellate females (2.5 vs. 4.1, 4.1, and 4.4 hours, respectively, after start of observation). This accounts for the peak of control female flight activity in the first interval but even so does not lend support to the hypothesis that ocelli are a factor in female flight activity because of the difference between shams and controls and the lack of one between shams and anocellates, although anocellate females on average did begin to fly later than shams. It was also found that sham males began flying significantly earlier than anocellate males, which began flying latest of all groups. Note that this correlates with a fact previously discussed, that in the first flight interval anocellate males flew least of all groups. There were no significant differences between control and sham males, or between controls and anocellates. The significant difference between sham and anocellate males, most closely related in terms of treatment, however, is important in indicating the presence of an ocellar effect among males initiating flight activity.

b. Flight cessation: The only significant differences ($p \leq 0.05$) found in times of flight cessation occurred between control males and females (10.2 vs. 10.5 hours after start of observation) and sham males and females (9.8 vs. 10.4 hours after start of observation), with the females continu-

ing to fly later than their male counterparts. Examination of Figure 10 shows that all the moths stopped flying at very nearly the same time. It is interesting to note, however, that whereas most of the standard deviations for mean cessation times were relatively small and fell within a small range of one another (control males +/- 3.61 h, control females +/- 2.54 h, sham males +/- 3.26 hours, sham females +/- 3.12 h, anocellate females +/- 3.71 h) that for anocellate males was relatively very large (+/- 18.1 h). This may be an indication that males, more than females, use their ocelli in regulating flight cessation. Note also that this phenomenon did not occur in flight initiation or duration.

c. Flight duration: This analysis, a function of the previous two (cessation minus initiation equals duration), indicated that control females flew significantly longer (6.1 hours, $p \leq 0.05$) than any other group. Sham females, however, did not fly significantly longer or shorter than anocellate females, and there were no significant differences among males at all, so it is not possible at this point to draw conclusions about a possible ocellar effect on flight duration in these moths.

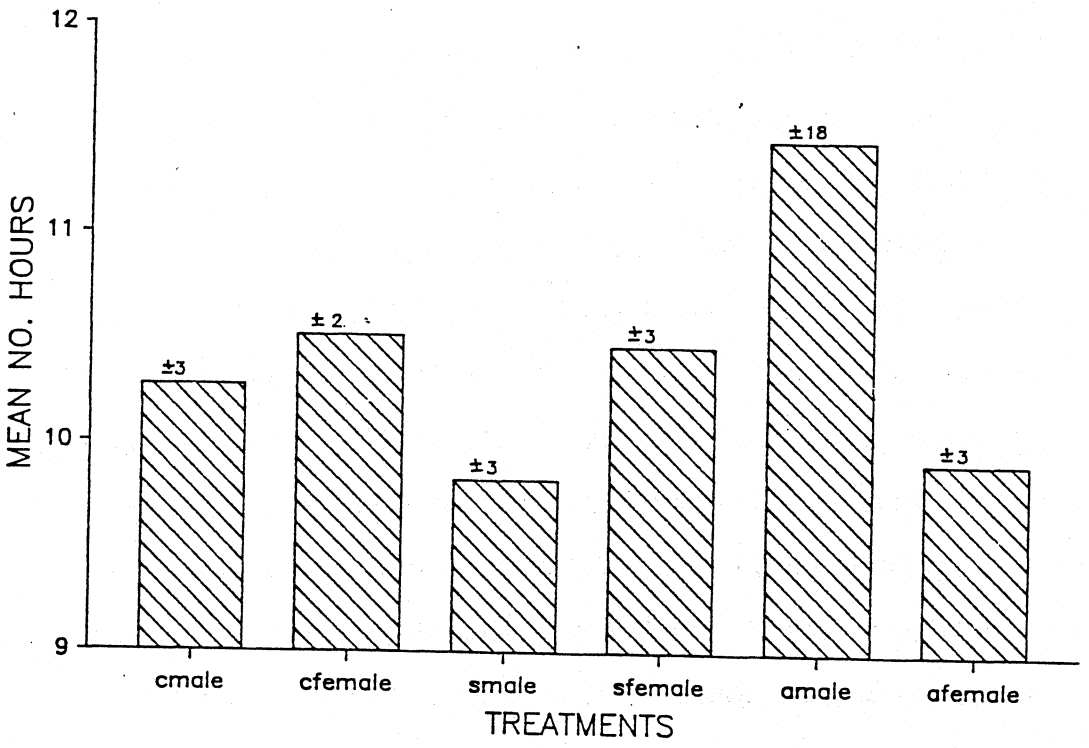


Figure 10. Mean Times of Flight Cessation.
Y-axis represents the mean number of hours after observation began (1/2 h before start of sunset). Numbers on top of the bars are standard deviations.

cmale = control males, n=91	a
cfemale = control females, n=87	b
smale = sham males, n=72	ac
sfemale = sham females, n=95	b
amale = anocellate males, n=53	ab
afemale = anocellate females, n=76	bc

Groups followed by the same letter are not significantly different (Kruskal-Wallis Analysis of Variance by Rank, $p \leq 0.05$).

B. Phase-Advance

In these experiments, male control, sham, and anocellate moths were placed in the actograph and allowed to habituate. Their flight activity was first monitored under normal conditions, then under a sunset that was advanced two hours. For this analysis, the mean time moths initiated flight was calculated for individual moths on individual days. Then the values for the days after sunset had been advanced were subtracted from the values for the day before the sunset had been advanced, and the resulting values analyzed. This meant that relative differences rather than absolutes were being analyzed. The Kruskal-Wallis test revealed no significant differences between groups, possibly because of the small sample size involved, so in order to reveal any biological significance that might be present the percentage of moths flying in the hours around sunset was plotted in Figures 11-14. Light intensity was superimposed onto the graphs.

It can be seen in Figure 11 that shams and controls began flying during sunset the day before sunset was advanced. The greatest increase in percentage of moths flying in these two groups occurred within the hour in which sunset was taking place. Although a greater

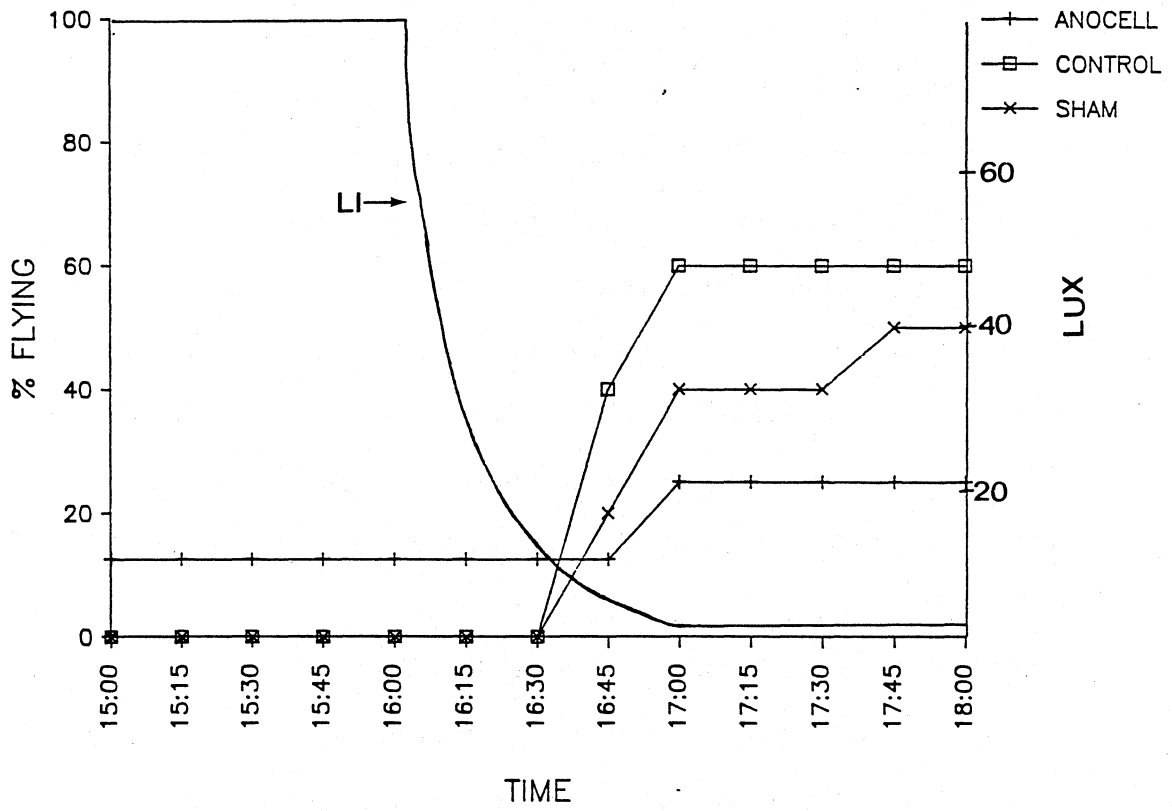


Figure 11. Percentage of Male Moths Flying the Day Before Phase-Advance.
Graph represents percentage of moths in each treatment group flying in the times indicated the day before sunset was advanced. (n=10, 10, 7 for controls, shams, and anocellates, respectively.)
Anocell = Anocellate moths.
LI = Light intensity. (in lux, right scale).

percentage of anocellate moths were flying by 16:00 (11%), by 17:00 a greater percentage of shams and controls were flying (shams 35%, controls 59% vs. anocellates 23%). This could be revealing; although Figure 6 indicates that sham and control males fly more than anocellates, it doesn't reveal the higher number of anocellate flights in the hour before sunset. This higher number could indicate a lack of precision with which anocellate moths initiate flight activity. The small sample size (n=10 controls, 10 shams, 7 anocellates) must be remembered, however; more repetitions would have to be made before conclusions could be drawn.

Phase-shifting sunset (Figure 12) results in a dramatic departure from the unshifted response seen in Figure 11. All groups initiated flight at the advanced sunset time, meaning they began flying at 14:30 rather than at 16:30 as in the previous figure. Again, the most rapid period of increase in percentage of moths flying occurred during the hour in which sunset was taking place. No group (except the one moth in the anocellate group) had begun flying at 15:00 the day before Phase-Advance (PA). All groups were flying by 15:00 the first day after PA, but shams and controls showed a greater percentage flying than anocellates (38% and 38% vs. 24%, respectively). Anocellate and control lines crossed at 16:00 (controls showed 38% flying and anocellates 48%),

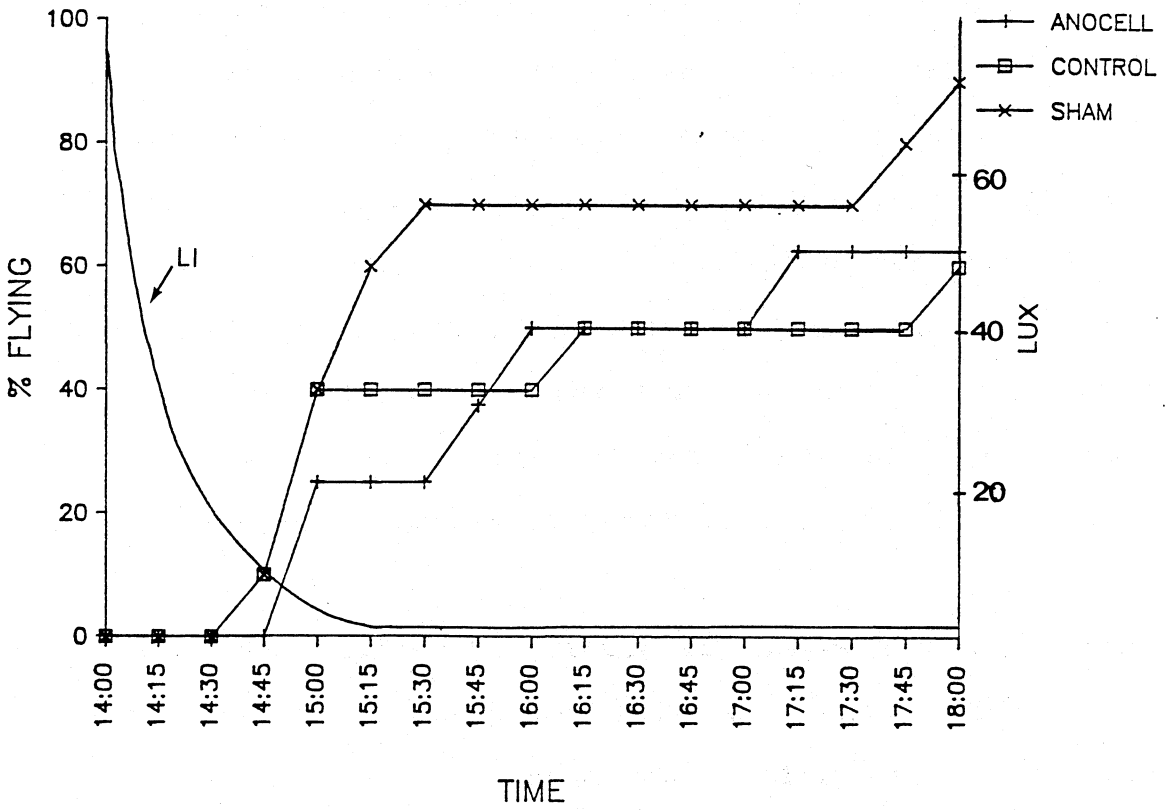


Figure 12. Percentage of Male Moths Flying the First Day After Phase-Advance.
Graph represents percentage of moths in each treatment group flying in the times indicated the first day after sunset was advanced. (n = 10, 10, 7 for controls, shams, anocellates, respectively).
Anocell = Anocellate moths.
LI = Light intensity (in lux, right scale).

then ran together from 16:15 to 17:00 at 48%. At 17:15 anocellates began showing a greater percent flying until at 18:00, the anocellates increased their percentage to 60% flying to be slightly greater than the controls with 58% flying. Sham moths consistently showed a greater percentage flying from 15:15 to 18:00. It would appear from Figure 12 that shams and controls responded to a greater degree to the advanced sunset, by greater numbers flying earlier, than anocellate moths. After sunset, however, controls and anocellates had similar percentages flying.

The second day after PA (Figure 13) shows a change from the first day after. The figure shows that shams still initiated flight shortly after sunset began (14:15) but that controls and anocellates didn't begin flying until about an hour later (15:15). This is probably more artificial than it is significant, since the greatest increase in flights within the sunset hour occurred closer to 15:00 than to 14:00. By 16:00 controls and shams both had a higher percentage of moths in flight than anocellates (70% and 70% vs. 35%, respectively).

Figure 14 shows that again the moths started flying during sunset, which had been reset to commence at its original time, 16:00, used 2 days previously. On the other days, flight began for all groups at least 30 minutes after sunset

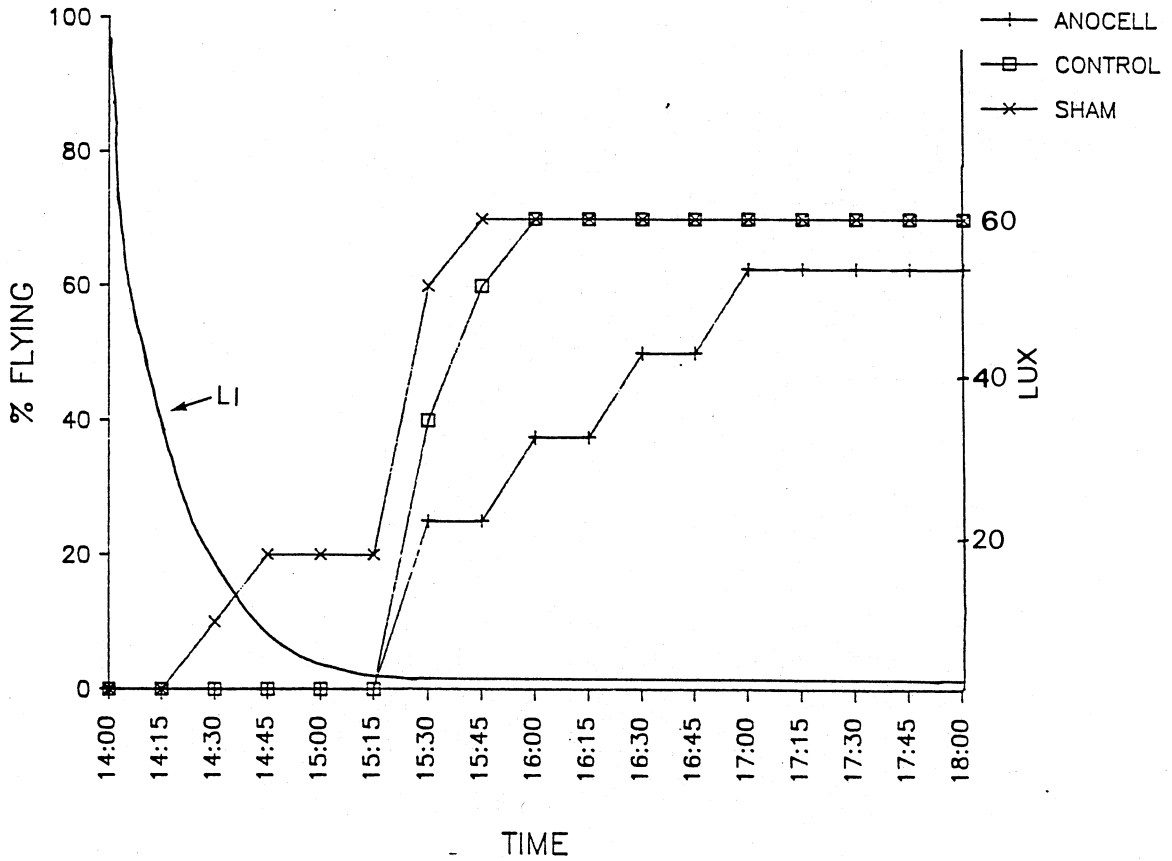


Figure 13. Percentage of Male Moths Flying the Second Day After Phase-Advance.
Graph represents percentage of moths in each treatment group flying in the times indicated the day before sunset was advanced. (n=10, 10, 7 for controls, shams, and anocellates, respectively.)
Anocell = Anocellate moths.
LI = light intensity (in lux, right scale).

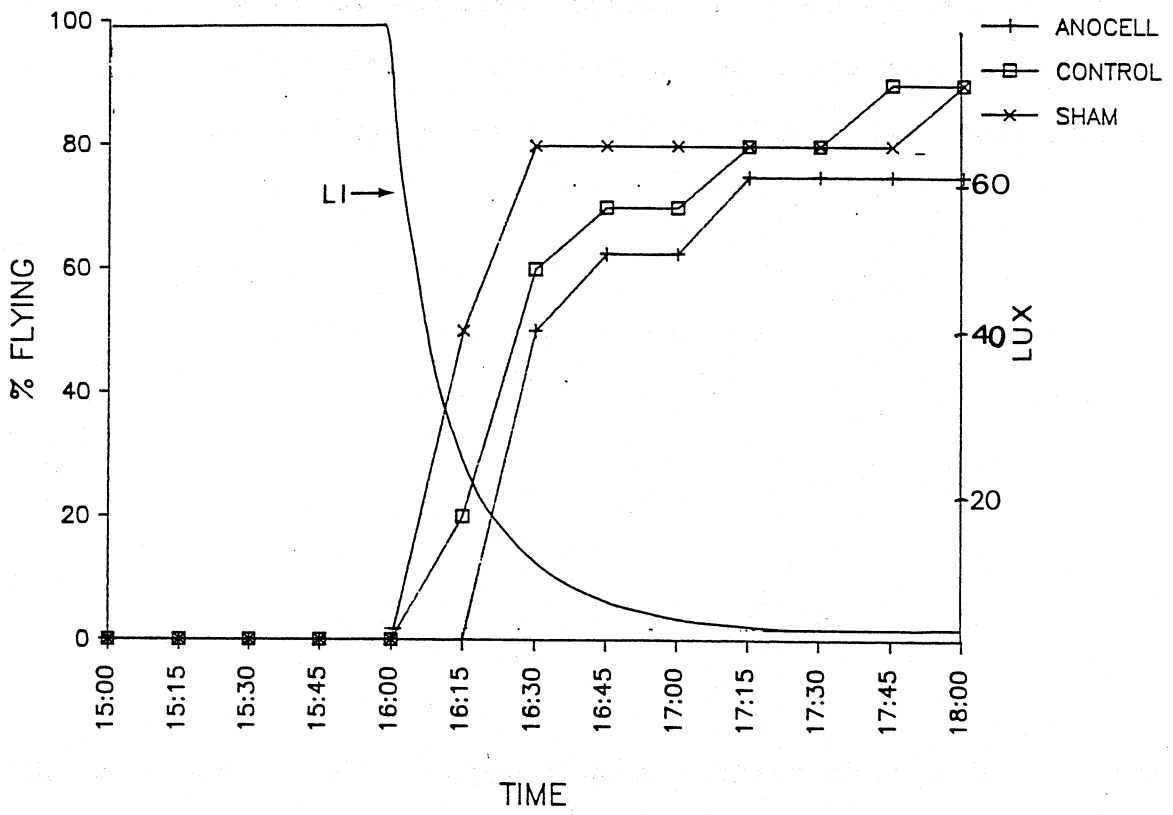


Figure 14. Percentage of Male Moths Flying the Day After Setback.
Graph represents percentage of moths in each treatment group flying in the times indicated the day after sunset was reset to its original time. (n= 10, 10, 7 for controls, shams, and anocellates, respectively.)
Anocell = Anocellate moths.
LI = light intensity (in lux, right scale).

had begun. On the day of setback, however, flight began for controls and shams within 15 minutes after sunset began, and for anocellates 30 minutes after. This could indicate that the advanced sunset of the previous two days predisposed the moths to begin flying earlier, but within the confines of sunset.

These data lead me to suggest with caution that both ocellate and anocellate male moths were able to adjust their flight timing to an advance in sunset, but that ocellate moths were able to do so more precisely than anocellate moths. It appears that when sunset was advanced a greater percentage of ocellate moths responded earlier than anocellate. The second day after the advance, only a slightly greater percentage of ocellate moths began flying earlier, indicating their adjustment to the advanced sunset was almost complete, but anocellate moths doubled their numbers flying early, indicating they were still attempting to adjust to the earlier sunset. These findings agree with those of Eaton et al

(1983) who conducted a similar experiment with moths with their ocelli occluded or ablated in the adult stage.

C. Free-Run

In these experiments, male control, sham, and anocellate moths were placed in the actograph and allowed to habituate. Their flight was monitored under normal conditions and under continuous darkness (DD). Again, Kruskal-Wallis testing ($p \leq 0.05$) revealed no significant differences between groups when analysis of relative differences was performed. For this analysis the mean number of hours after monitoring began at 15:30 (i.e., 1/2 h before sunset started) that moths initiated flight was calculated for individual moths on individual days. Then the values for the days after DD had been established were subtracted from the values for the day before DD had been established. The lack of significant difference could be attributable to the relatively small sample size (n=10 controls, 10 shams, 9 anocellate). Figures 15-19 are plots of percentages of moths in treatment groups flying within the first 6 hours after monitoring began.

Before DD was established (Figure 15) no moths in any of the groups began flying during the first hour. By the end of the sixth hour the percentages of sham and control moths flying

were identical (68%) with a lower percentage of anocellates flying (55%).

Figure 16 represents the data on the first day after DD was established; the moths here had no sunset to cue them to fly. This figure appears much the same as Figure 15. The greatest increase in percentage of moths flying occurred between the first and second hours (from 0% flying at 16:30 to 54% of shams, 60% of controls, and 31% of anocellates flying at 21:30) so it appears all groups were still initiating flight probably within the second hour, certainly before the end of the third hour. The second day after DD establishment (Figure 17) reveals what would appear to be greater confusion among the moths; controls appear to initiate flight after the 18:30, and it is difficult to see one distinct initiation point with shams and anocellates.

It is my contention that the small sample size magnifies individual variability in this experiment so it would be almost impossible to find these moths' free-running flight activity rhythm, and therefore impossible to locate relative differences in it between treatment groups. It is interesting to observe, however, the flight behavior of the moths

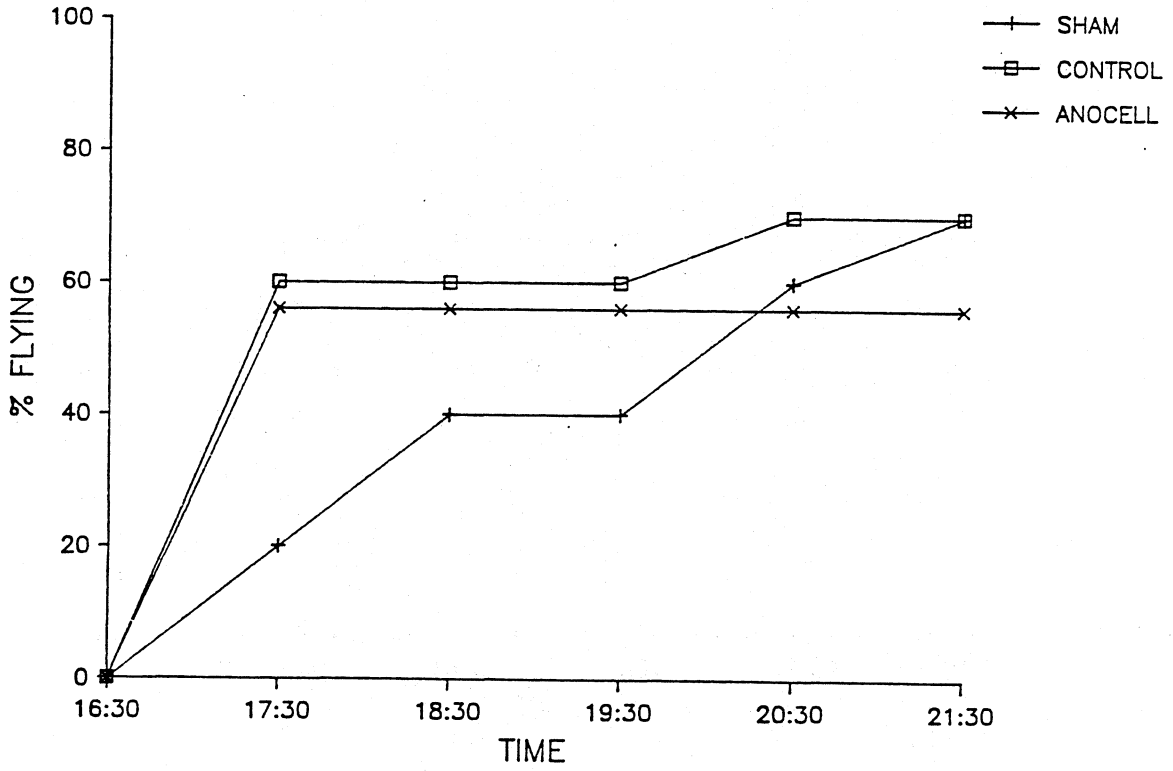


Figure 15. Percentage of Male Moths Flying the Day Before DD was Established.
Graph represents percentage of moths in each treatment group flying within the first six hours after observation began the day before DD was established. (n=10, 10, 9 for controls, shams, and anocellates, respectively.)
Anocell = Anocellate moths.

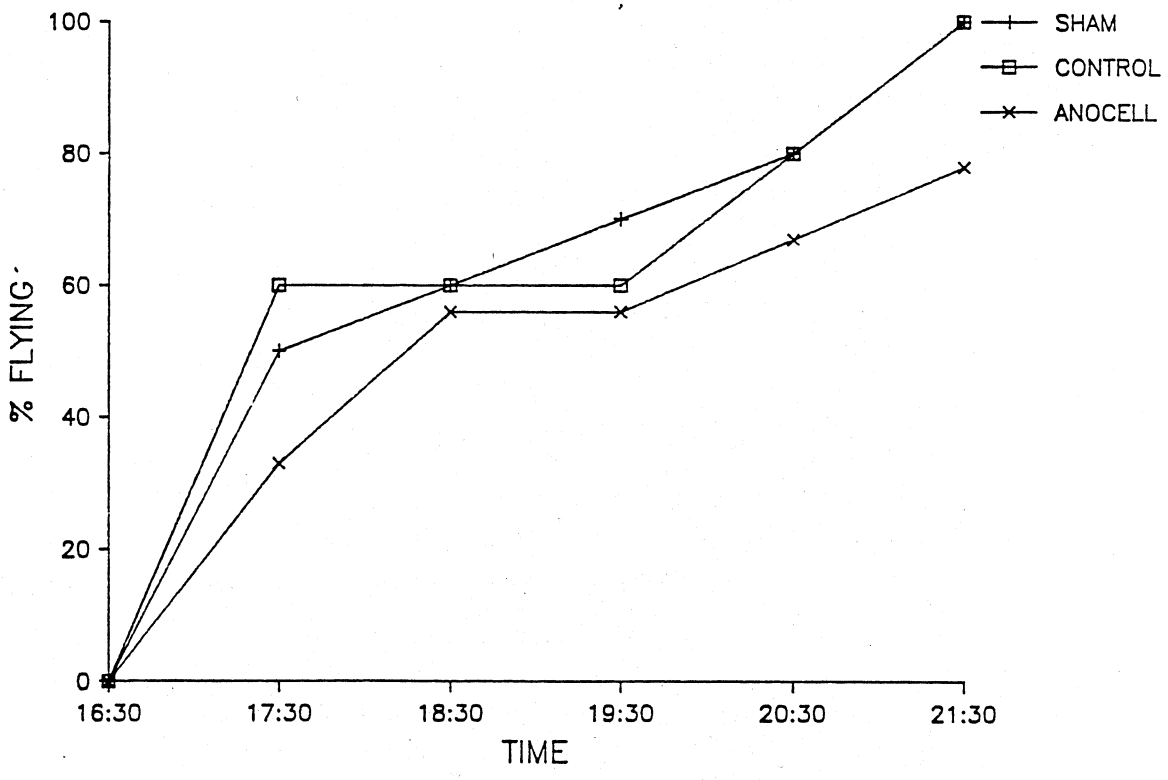


Figure 16. Percentage of Male Moths Flying Day 1 After DD was Established.
Graph represents percentage of moths in each treatment group flying within the first six hours after observation began the day after DD was established. (n=10, 10, 9 for controls, shams, and anocellates, respectively.)
Anocell = Anocellate moths.

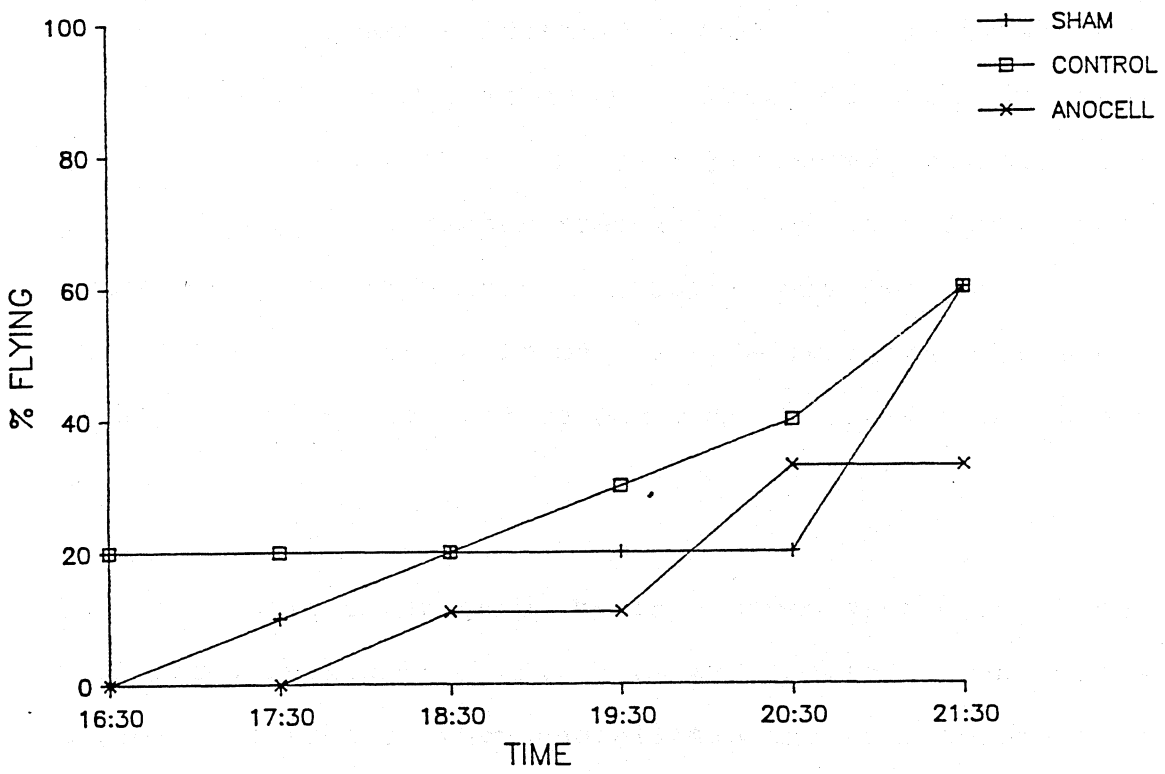


Figure 17. Percentage of Male Moths Flying Day 2 After DD was Established.
Graph represents percentage of moths in each treatment group flying within the first six hours after observation began the second day after DD was established. (n=10, 10, 9 for controls, shams, and anocellates, respectively.)
Anocell = Anocellate moths.

when a LD cycle is re-established (Figures 18 and 19). Immediately (Figure 18) an initiation point for all 3 groups is evident after the third hour. This initiation point shifts to a point after the first hour the second day after LD re-establishment (Figure 19); that is where it was before DD (Figure 15), and that time corresponds with the time of sunset. In Figure 19 the maximum percentage of moths flying for all groups is higher than in Figure 15 (90% vs. 68% for shams, 80% vs. 68% for controls, and 65% vs. 55% for anocellates). This could be a simple function of age of the moths, or it could be a response to the re-establishment of LD.

The only differences shown by this experiment were those established previously - that sham and control moths fly with a greater intensity than anocellates and that constant darkness upsets the flight activity rhythms of these moths. Although no relative differences between treatment groups were found, it is meaningful that anocellate moths were also affected by the imposition of DD. This indicates that the compound eyes, in addition to the ocelli, were involved in entrainment of the rhythm to light, supporting the findings of previous researchers (Nowosielski and Patton 1963, Wellington 1974, Rivault 1976, Fent and Wehner 1985).

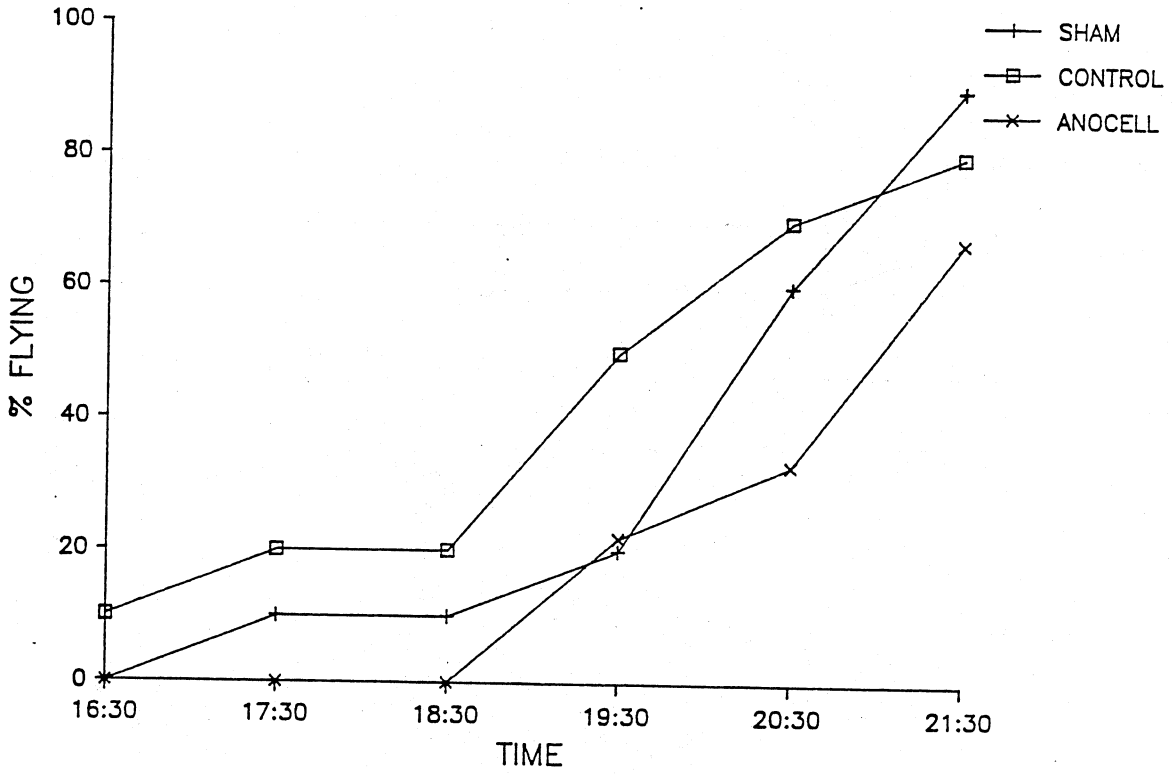


Figure 18. Percentage of Male Moths Flying Day 1 After Return to LD.
Graph represents percentage of moths in each treatment group flying within the first six hours after observation began the first day after LD was re-established. Sunset began at 16:00. (n=10, 10, 9 for controls, shams, and anocellates, respectively). Anocell = Anocellate moths.

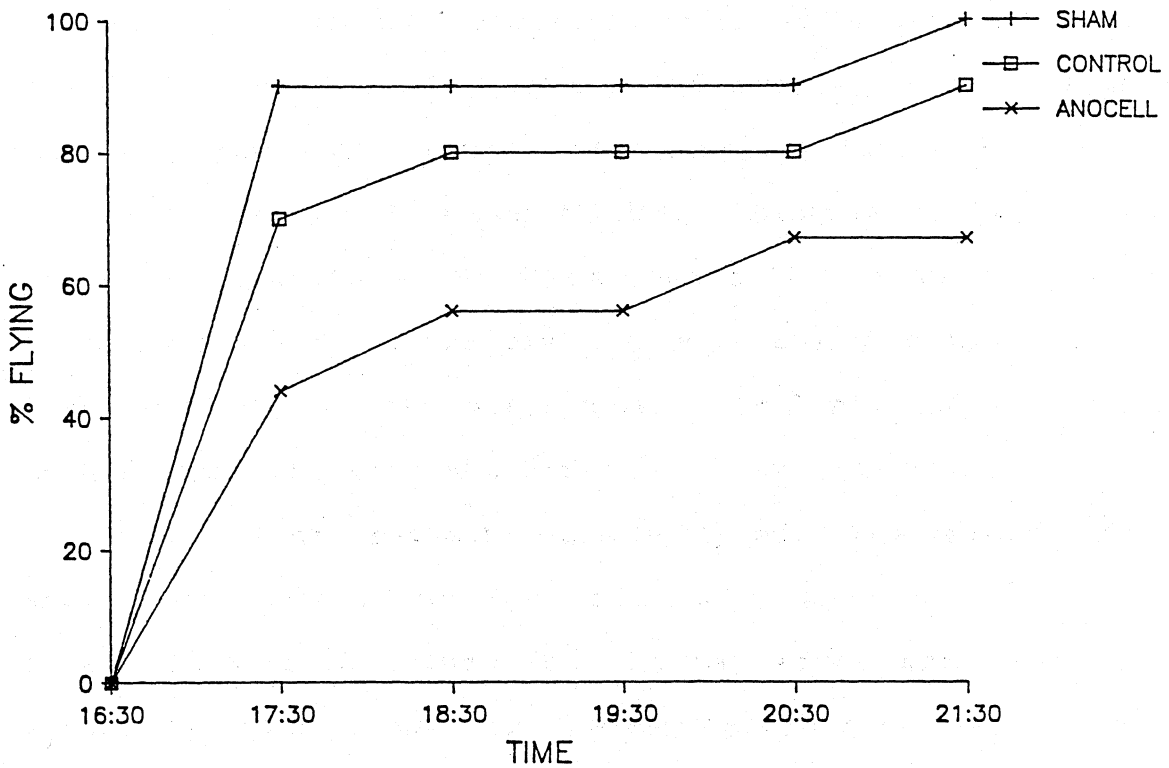


Figure 19. Percentage of Male Moths Flying Day 2 After Return to LD.
Graph represents percentage of moths in each treatment group flying within the first six hours after observation began the second day after LD was re-established. Sunset began at 16:00. (n=10, 10, 9 for controls, shams, anocellates, respectively.) Anocell = Anocellate moths.

II. OCTOPAMINE AND FLIGHT ACTIVITY

These experiments involved monitoring the flight activity of female control, sham, and anocellate moths before and after topical application of DMSO alone and octopamine dissolved in DMSO. The Kruskal-Wallis test ($p \leq 0.05$) revealed no significant differences between treated and untreated moths when the following data were analyzed: time of flight initiation, mean total numbers of flights, and mean numbers of flights in the first three hours after application. As in the Phase-Advance and Free-Run studies, relative differences in the above parameters were examined rather than the values themselves. Since it was previously established that significant differences did exist between ocellate and anocellate moth flight behavior, the differences within groups between before and after treatment were analyzed. The values listed above were calculated for individual moths on individual days. Then those values for the day after treatment were subtracted from those for the day before treatment. Here again, sample sizes were small and standard deviations extremely large (see Tables 2 and 3).

In view of the above it was thought it would be useful to plot percentage flights as described previously, to see if differences existed that weren't being revealed by conventional analysis. Figure 20 seems to reveal a difference,

Table 2. Mean number of flights for treatment groups

Treatment	n	Mean[1]	Standard Deviation[2]
C-N[3]	9	5.11	120.63
C-DMSO	10	84.50	254.82
C-10%	12	-5.00	33.78
S-N	9	-16.44	112.50
S-DMSO	9	-23.44	56.69
S-10%	11	-3.73	29.92
A-N	9	15.78	49.04
A-DMSO	9	9.56	21.70
A-10%	12	83.25	244.60

[1]Values represent relative differences in flight numbers before and after treatment. In some cases, this resulted in negative values for means.

[2]Standard deviations were calculated by the SAS MEANS Procedure (SAS Institute, Inc., 1982).

[3]Abbreviations: C- Control, S- Sham, A- Anocellate; -N no treatment, -DMSO DMSO only, -10% 10% octopamine.

Table 3. Mean flight initiation times for treatment groups

Treatment	n	Mean[1]	Standard Deviation[2]
C-N[3]	9	0.33	2.21
C-DMSO	10	2.55	5.36
C-10%	12	0.17	1.87
S-N	9	0.17	1.04
S-DMSO	9	1.17	5.65
S-10%	11	-0.25	4.70
A-N	9	1.92	3.36
A-DMSO	9	-0.19	4.57
A-10%	12	0.73	2.28

[1]Values represent relative differences in the number of hours after observation began (at 15:30) that moths initiated flight before and after treatment. In some cases, this resulted in negative values for means.

[2]Standard deviations were calculated by the SAS MEANS Procedure (SAS Institute, Inc., 1982).

[3]Abbreviations: C- Control, S- Sham, A- Anocellate; -N no treatment, -DMSO DMSO only, -10% 10% octopamine.

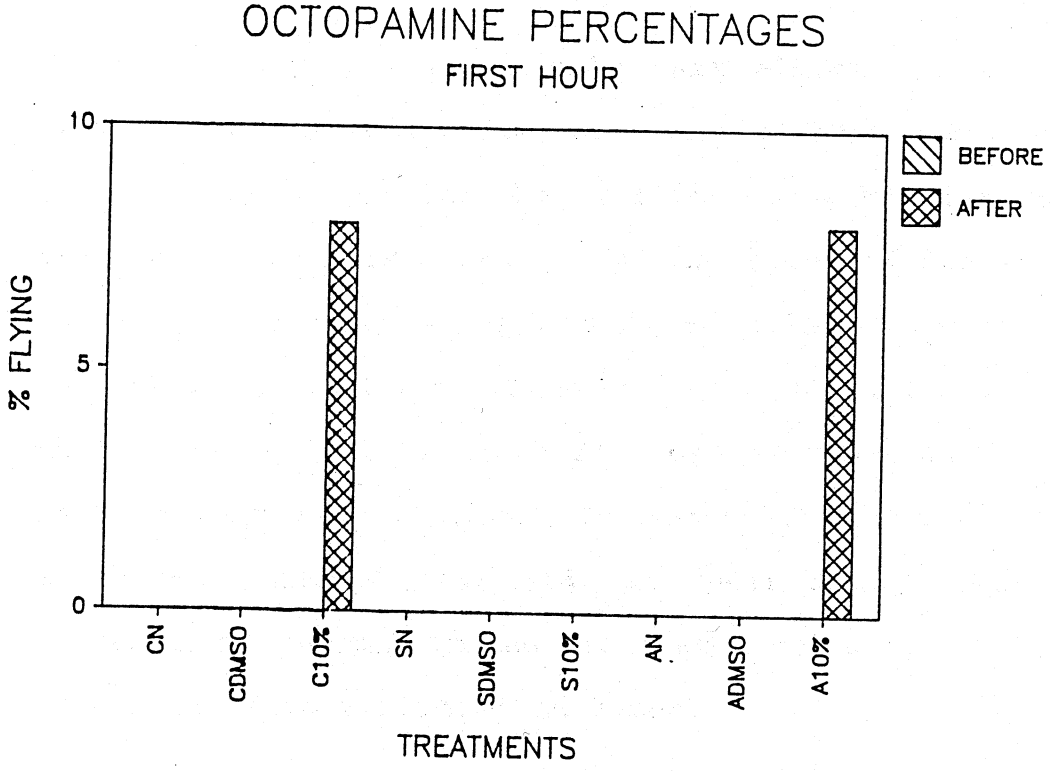


Figure 20. Percentage of Moths Flying Within the First Hour Before/After Treatment.

Abbreviations:
CN=control, no treatment
CDMSO=control, DMSO
C10%=control, 10% octopamine
SN=sham, no treatment
SDMSO=sham, DMSO
S10%=sham, 10%
AN=anocellate, no treatment
ADMSO=anocellate, DMSO
A10%=anocellate, 10% octopamine.

but it must be noted that 8%, the value of the peaks shown for control-10% and anocellate-10% treatments, of 12, the sample size for those treatments, is not quite 1 (0.96), so its relevance is negligible.

Figures 21 and 22 reveal only that almost all treatments, including those groups that were left untreated and that were treated with DMSO alone, began flying somewhat earlier after treatment. This is most likely a function of the age of the moths; it was shown in previous unpublished studies by Eaton that flight activity patterns change as the moths age, namely that moths up to 5 days old fly more as they age. After 5 days, flight numbers begin to decline. In this study the moths were under 5 days old, so their flight was probably increasing - perhaps in the early evening, which might result in earlier flight initiation times.

In a preliminary experiment to see how quickly DMSO carries substances across the moth cuticle, 100 µg chlordimeform (95% a.i. in Galecron® obtained from Ciba-Geigy) dissolved in 1 µl DMSO was applied to the dorsal thorax, which was free of scales, of each of 10 cabbage looper moths. Within 15 minutes the toxic effects of chlordimeform were manifested by greatly increased flight activity, eventually followed by other chlordimeform symptoms and death. The similar structures of chlordimeform and octopamine (Figure

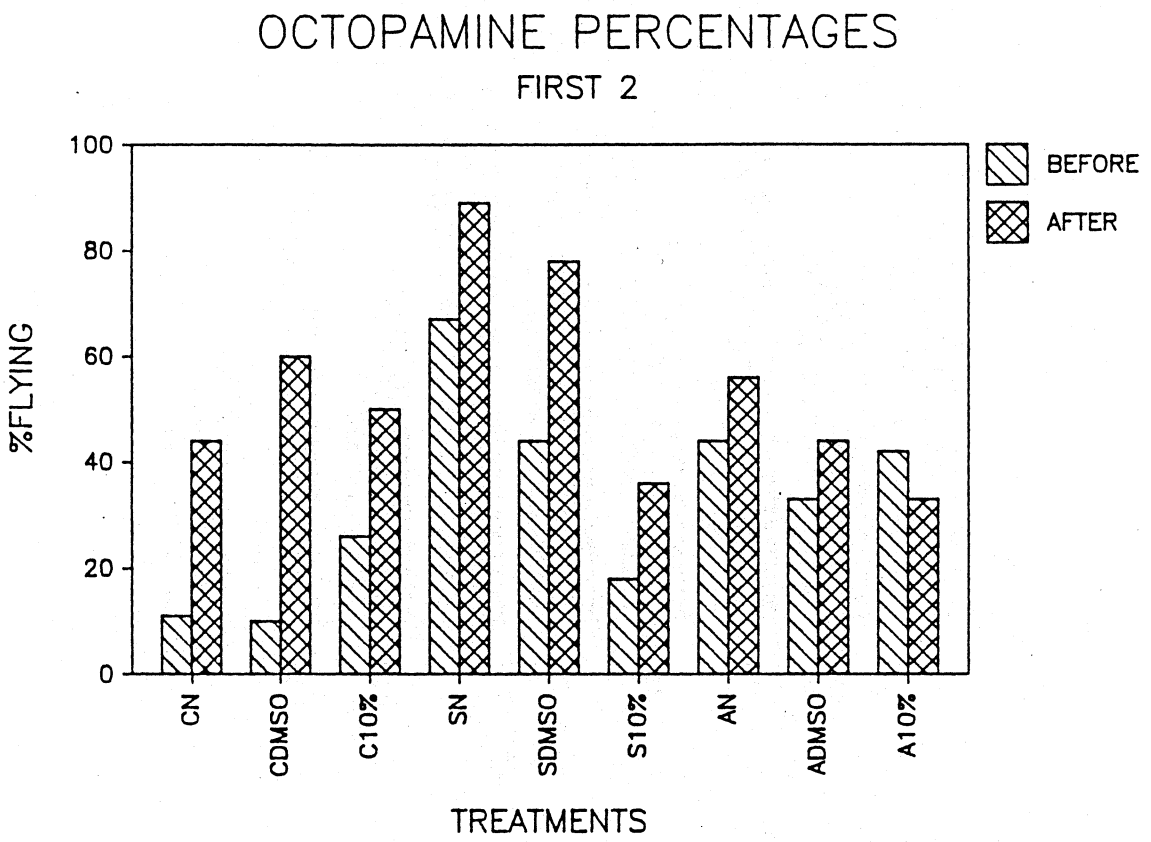


Figure 21. Percentage of Moths Flying Within the First Two Hours Before/After Treatment.

Abbreviations: CN=control, no treatment
CDMSO=control, DMSO
C10%=control, 10% octopamine
SN=sham, no treatment
SDMSO=sham, DMSO
S10%=sham, 10% octopamine
AN=anocellate, no treatment
ADMSO=anocellate, DMSO
A10%=anocellate, 10% octopamine.

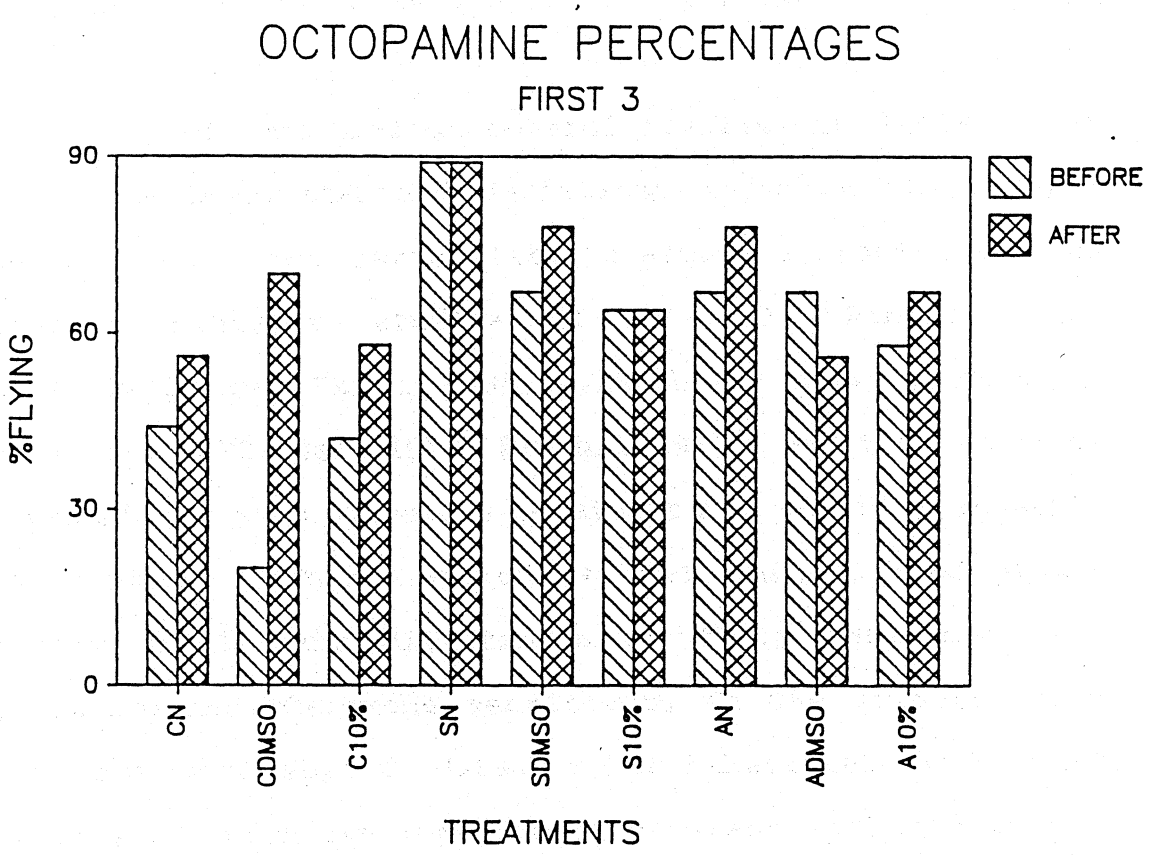


Figure 22. Percentage of Moths Flying Within the First Three Hours Before/After Treatment.

Abbreviations: CN=control, no treatment
CDMSO=control, DMSO
C10%=control, 10% octopamine
SN=sham, no treatment
SDMSO=sham, DMSO
S10%=sham, 10% octopamine
AN=anocellate, no treatment
ADMSO=anocellate, DMSO
A10%=anocellate, 10% octopamine.

1), lead to the conclusion that octopamine as used in this experiment readily penetrated the moth's cuticle in about 15 minutes. Why then were no effects similar to those of its antagonist manifested at significant levels?

The first and most obvious possibility lies in Tables 2 and 3, in the large standard deviations found in these experiments. Individual variability is always a problem in quantitative behavior studies (Lutz 1932, Roberts 1960, Nowosielski and Patton 1963, Leuthold 1966, Lipton and Sutherland 1970, Ball 1972, Rivault 1976), and this study was no exception, even given the sophisticated activity-measuring device used. Linn and Roelofs (1984) did not find statistically significant differences in their experiment with octopamine and pheromone sensitivity in the oriental fruit moth, but were able to detect other behavioral differences, the like of which are impossible to detect with our system. One way of decreasing variability in the study might be to avoid the behavioral changes associated with aging by using one set of moths to establish base-line data, and a new set of moths with ages identical to the previous set to treat. This, of course, at least doubles the labor-intensity of each experiment. Another way to increase reliability is to increase sample size. Judging from my data, sample size would need to be increased enormously before significant differences might be found.

A different answer to the question raised above is that octopamine alone does not affect the circadian rhythm of flight activity in cabbage looper moths. Linn and Roelofs (1985) recently reported studies that indicate this might be the case. They found no change in male cabbage looper moth flight activity under normal scotophase conditions when octopamine was applied by injection, except at very high levels, at which paralysis was observed. They did however find differences in the moths' response to a pheromone plume when the moths were injected with octopamine. This enhancement of a behavioral response to a specific stimulus has also been found in blowfly feeding in response to tarsal stimulation (Long and Murdock, 1983). In their study, Linn and Roelofs did observe that serotonin increased the male cabbage looper moth's level of flight activity in the absence of pheromone.

The results of these studies indicate that ocelli are involved in the timing and intensity of cabbage looper moth flight activity in a number of ways. When ocelli were absent moths were less accurate in their timing of flight initiation and flew less than when ocelli were present. No evidence was found that supports the theory that octopamine restores normal flight activity to anocellate moths, or in fact increases spontaneous flight activity at all, when it is topically applied.

SUMMARY

The results obtained in pursuing the first objective, confirmation of the role of ocelli in cabbage looper moth flight activity, indicated the following:

1. Male moths with ocelli flew more than male moths without. The period of greatest male moth activity occurred near the middle of the night.

2. Male moths in general flew more than female moths.

3. Female moths flew more in the early part of the evening than males.

4. The cauterization operation itself appeared to affect flight activity by decreasing numbers of flights. This effect became less significant as numbers of flights increased and was therefore less obvious among males than among females.

5. Male moths with ocelli appeared to respond to a smaller change in light intensity than anocellates, by initiating flight earlier in the evening.

6. Females continued to fly later into the morning than males.

7. Male moths both with and without ocelli were able to adjust to a 2-hour advance in the time of sunset, but moths with ocelli appeared to do so more accurately.

8. Constant darkness disturbed the flight activity rhythm of male moths. Resetting LD reestablished the rhythm for all groups. Results imply involvement of both the ocelli and the compound eyes in entrainment of the activity rhythm.

9. The greatest stumbling block to studies of this kind is individual variability which can mask biological differences and statistical significance. In future studies, all possible steps should be taken to reduce factors contributing to variability.

Pursuit of the second objective, the study of the relationship between octopamine, flight activity, and the presence or absence of ocelli, resulted in the following: no statistically significant differences were observed between octopamine-treated and untreated moths with and without ocelli. No other consistent differences were observed. This led to the suggestion that perhaps octopamine alone does not

affect spontaneous flight activity in these moths. Further experimentation with octopamine and with other biogenic amines and amine agonists and antagonists is encouraged.

REFERENCES

Bailey, B. A., R. J. Martin, and R. G. H. Downer. 1983. Haemolymph octopamine levels during and following flight in the American cockroach, Periplaneta americana L. Can. J. Zool. 62:19-22.

Ball, H. J. 1965. Photosensitivity in the terminal abdominal ganglion of P. americana (L.). J. Insect Physiol. 11:1311-1315.

1972. Photic entrainment of circadian activity rhythms by direct brain illumination in the cockroach Blaberus craniifer. J. Insect Physiol. 18:2449-2455.

Bodnaryk, R. P. 1980. Changes in brain octopamine levels during metamorphosis of the moth, Mamestra configurata Wlk. Insect Biochem. 10:169-173.

Byers, J. A. and M. A. Unkrich, 1983. Electronic light intensity control to simulate dusk and dawn conditions. Ann. Entomol. Soc. Am. 76:556-558.

Bullock, T. H. and G. A. Horridge 1965. Structure and function in the nervous system of invertebrates vol. VI and VII, W. H. Freeman and Co., San Francisco, London.

Caesar, C. J. 1973. Die Stirnaugen der Ameisen. Zool. Jb. (Anat.) 35:161-242.

Chapman, R. F. 1982. The insects: structure and function 3rd ed. Harvard University Press, Cambridge, MA.

Cloudsley-Thompson, J. L. 1953. Studies on diurnal rhythms. III. Photoperiodism in the cockroach, Periplaneta Americana (L.) Ann. Mag. Nat. Hist. 6:705-712.

Cornwell, P. B. 1955. The functions of the ocelli of Calliphora (Diptera) and Locusta (Orthoptera). J. Exp. Biol. 32:217-237.

Davenport, A. P. and P. D. Evans. 1984a. Changes in haemolymph octopamine levels associated with food deprivation in the locust Schistocerca gregaria Physiol. Entomol. 9:269-274.

1984b. Stress-induced changes in octopamine levels of insect haemolymph. Insect Biochem. 14(2):135-143.

David, J. C. and H. Verron. 1982. Locomotor behavior in relation to octopamine levels in the ant Lasius niger. Experientia 38:650-651.

Dickens, J. C., J. L. Eaton. 1973. External ocelli in Lepidoptera previously considered to be anocellate. Nature 242:205-206.

Dow, M. A. and J. L. Eaton. 1976. Fine structure of ocelli of the cabbage looper moth (Trichoplusia ni). Cell Tissue Res. 171:523-533.

Dowling, J. E. and R. L. Chappell. 1972. Neural organization of the median ocellus of the dragonfly, II. Synaptic structure. J. Gen. Physiol. 60:148-165.

Dumortier, B. 1972. Photoreception in the circadian rhythm of stridulatory activity in Ephippiger (Ins., Orthoptera). Likely existence of two photoreceptive systems. J. comp. Physiol. 77:80-112.

Eaton, J. L. 1971. Insect photoreceptors: An internal ocellus is present in sphinx moths. Science 173:822-823.

1975. Electroretinogram components of the ocellus of the adult cabbage looper moth, Trichoplusia ni. J. Insect Physiol. 21:1511-1515.

1976. Spectral sensitivity of the ocelli of the adult cabbage looper moth. J. Comp. Physiol. 109:17-24.

1983. Identification of dorsal ocellar primordia in larvae of Trichoplusia ni (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Am. 76(5):884-886.

1985. Actograph studies of light effects on Trichoplusia ni flight activity. pp. 233-240. In: Gewecke, M. and G. Wendler (eds.) Proc. Symp. 4.5 XVII. Int. Congress Entomol. Paul Parey, Berlin, Hamburg.

Eaton, J. L. and L. G. Pappas. 1977. Synaptic organization of the cabbage looper moth ocellus. Cell Tissue Res. 183:291-297.

Eaton, J. L., K. R. Tignor, and G. I. Holtzman. 1983. Role of moth ocelli in timing flight initiation at dusk. Physiol. Entomol. 8:371-375.

Ebihara, S. K. Uchiyama, and I. Oshima. 1984. Circadian organization in the pigeon, Columba livia: The role of the pineal organ and the eye. J. Comp. Physiol. A 154:59-69.

Erspramer, V. and G. Boretti. 1951. Identification and characterisation by paper chromatography of enteramine, octopamine, tyramine, histamine, and allied substances in extracts of posterior salivary glands of Octopoda and in other tissue extracts of vertebrates and invertebrates. Arch. Int. Pharmacodyn. 88:296-332.

Evans, P. D. 1980. Biogenic amines in the insect nervous system. pp. 317-474. In: Berridge, M. J., J. E. Treherne, and V. B. Wigglesworth, eds. Advances in Insect Physiology vol. 15. Academic Press, Inc. (London) Ltd.

Fent, K. and R. Wehner. 1985. Ocelli: a celestial compass in the desert ant Cataglyphis. Science 228:192-194.

Fowler, D. J. and C. J. Goodnight. 1966. Neurosecretory cells: Daily rhythmicity in Leiobunum longipes. Science 152:1078-1080.

Fowler, D. J., C. J. Goodnight, and M. M. LaBrie. 1972. Circadian rhythms of 5-hydroxytryptamine (serotonin) production in larvae, pupae, and adults of Drosophila melanogaster (Diptera: Drosophilidae). Ann. Entomol. Soc. Am. 65(1):138-141.

Geispitz, K. F. 1957. The mechanism of acceptance of light stimuli in the photoperiodic reaction of Lepidoptera larvae. Zool. Zh. 36:548-560.

Godden, D. H. 1973. A reexamination of circadian rhythmicity in Carausius morosus. J. Insect Physiol. 19:1377-1386

Goodman, C. S. 1974. Anatomy of locust interneurons: constancy and variability. J. Comp. Physiol. 95:185-201.

Goodman, L. J. 1970. The structure and function of the insect dorsal ocellus. Adv. Insect Physiol. 7:97-195.

1975. The neural organization and physiology of the insect dorsal ocellus. In: G. A. Horridge, ed. The compound eye and vision of insects Editor. Oxford University Press, Oxford.

1981. Organization and physiology of the insect dorsal ocellar system. In: H. Autrum, ed. Handbook of sensory physiology, vol. VII/6C, Comparative physiology and evolution of vision in invertebrates. Springer Verlag, New York.

Goodman, L. J., J. A. Patterson, and P. G. Mobbs. 1975. The projections of ocellar neurons within the brain of the

locust, Schistocerca gregaria. Cell Tissue Res. 57:467-492.

Goodman, L. J., P. G. Mobbs, and J. B. Kirkham. 1979. The fine structure of the ocelli of Schistocerca gregaria: The neural organization of the synaptic plexus. Cell Tissue Res. 196:487-510.

Goosey, M. W. and D. J. Candy. 1980. The D-octopamine content of th locust Schistocerca americana gregaria and its elevation during flight. Insect Biochem. 10:393-397.

1982. The release and removal of octopamine by tissues of the locust Schistocerca americana gregaria. Insect Biochem 12(6):681-685.

Harker, J. E. 1955. Control of diurnal rhythms of activity in Periplaneta americana L. Nature, Lond. 175:733.

1956. Factors controlling the diurnal rhythm of activity in Periplaneta americana L. J. Exp. Biol. 33:224-234.

Henneberry, T. J. and A. N. Kishaba. 1966. Cabbage loopers. pp. 461-478. In: C. N. Smith, ed. Insect colonization and mass production. New York: Academic Press pp. 461-478.

Hinks, C. F. 1967. Relationship between serotonin and the circadian rhythm in some nocturnal moths. Nature 214:386-387.

Homann, H. 1924. Zum Problem der Ocellenfunktion bei den Insekten. Z. Vergl. Physiol. 1:541-578.

Hoyle, G. 1975. Evidence that insect dorsal unpaired median (DUM) neurones are octopaminergic. J. Exp. Zool. 193:425-431.

Hoyle, G., D. Dagan, B. Moberly, and W. Colquhoun. 1974. Dorsal unpaired median insect neurons make neurosecretory endings on skeletal muscle. J. Exp. Zool. 187:159-165.

Hoyle, G. and D. L. Barker. 1976. Synthesis of octopamine by insect dorsal median unpaired neurons. J. Exp. Zool. 193:433-439.

Jacklet, J. W. 1985. Neurobiology of circadian rhythms generators. Trends in Neurosci. 8(2):69-73.

Jahagirdar, A. P., R. G. H. Downer, and T. Viswanatha. 1984. Influence of octopamine on trehalose activity in muscle

and hemolymph of the American cockroach, Periplaneta americana L. Biochimica et Biophysica Acta 801:177-183.

Kruskal, W. H. 1952. A nonparametric test for the several sample problem. Ann. Math. Stat. 23:525-540.

Lees, A. D. 1960a. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid Megoura viciae Buckton - II. The operation of the "interval times" in young clones. J. Insect Physiol. 4:154-175.

1960b. Some aspects of animal photoperiodism. Cold Spring Harb. Symp. Quant. Biol. 25:261-268.

1964. The location of the photoperiodic receptors in the aphid Megoura viciae. J. Exp. Biol. 41:119-133.

Leuthold, R. 1966. Die Bewegungsaktivitat der weiblichen Schabe Leucophaea maderae (F.) im Laufe des Fortpflanzungszyklus und ihre experimentelle Beeinflussung. J. Insect Physiol. 12:1303-1331.

Lillywhite, P. G. 1978. Coupling between locust photoreceptors revealed by a study of quantum bumps. J. Comp. Physiol. 125:13-27.

Lindauer, M. and B. Schricker. 1963. Uber die Funktion der Ocellen bei den Dammerungsflugen der Honigbiene. Biol. zentralbl. 82:721.

Linn, C. E. Jr. and W. L. Roelofs. 1984. Sublethal effects of neuroactive compounds on pheromone response thresholds in male oriental fruit moths. Arch. Insect Biochem. and Physiol. 1:331-344.

1985.Neuropharmacological effects on olfactory perception. In: T. Payne and M. Birch, eds. Mechanisms of perception and orientation in insects to olfactory signals. NSF-NATO seminar, Oxford Press, Oxford.

Lipton, G. R. and D. J. Sutherland. 1970. Activity rhythms in the American cockroach, Periplaneta Americana J. Insect Physiol. 16:1555-1556.

Loher, W. 1972. Circadian control of stridulation in the cricket Teleogryllus commodus Walker. J. Comp. Physiol. 79:173-190.

Loher, W. and M. K. Chandrashekaran. 1970. Circadian rhythmicity in the oviposition of the grasshopper Chorthippus curtipennis J. Insect Physiol. 16:1677-1688.

Long, T. F. and L. L. Murdock. 1983. Stimulation of blowfly feeding behavior by octopaminergic drugs. Proc. Natl. Acad. Sci. USA 80:4159-4163.

Lutz, F. E. 1932. Experiments with Orthoptera concerning diurnal rhythms. Am. Mus. Novitates 550:1-24.

Mercer, A. R., P. G. Mobbs, A. P. Davenport, and P. D. Evans. 1983. Biogenic amines in the brain of the honeybee, Apis mellifera. Cell Tissue Res. 234:655-677.

Mobbs, P. G. 1978. Some aspects of the structure and development of the locust ocellus. PhD. Thesis, University of London.

Nishiitsutsuji-Uwo, J. and C. S. Pittendrigh. 1968. Central nervous system control of circadian rhythmicity in the cockroach. II. The pathway of light signals that entrain the rhythm. Z. vergl. Physiol. 58:1-13.

Nowosielski, J. W. and R. L. Patton. 1963. Studies on circadian rhythms of the house cricket Gryllus domesticus L. J. Insect Physiol. 9:401-410.

Orchard, I. 1982. Octopamine in insects: neurotransmitter, neurohormone, and neuromodulator. Can. J. Zool. 60(4):659-669.

Orchard, I., J. A. Carlisle, B. G. Loughton, J. W. D. Gole, and R. G. H. Downer. 1982. In vitro studies on the effects of octopamine on locust fatbody. Gen. Comp. Endocrinol. 48: 7-13.

Orchard, I. and A. B. Lange. 1984. Cyclic AMP in locust fat body: correlation with octopamine and adipokinetic hormones during flight. J. Insect Physiol. 30(12):901-904.

Page, T. L. 1985. Circadian organization in cockroaches: Effects of temperature cycles on locomotor activity. J. Insect Physiol. 31(3):235-242.

Parry, D. A. 1947. The function of the insect ocellus. J. Exp. Biol. 24:211-219.

Pimley, R. W. 1984. Effects of biogenic amines on fat cells of Glossina morsitans in vitro. J. Insect Physiol. 30(7):587-590.

Plotnikova, S. I. 1969. Effectory neurones with several axons in the ventral nerve cord of Locusta migratoria. J. Evol. Biochem. Physiol. 5:339-341.

Renner, M. and T. Heinzeller. 1979. Do trained honeybees with reliably blinded ocelli really return to the feeding site? J. Apicult. Res. 18(3):225-229.

Rensing, L. 1964. Daily rhythmicity of corpus allatum and neurosecretory cells in Drosophila melanogaster (Merg.). Science (Wash.) 114:1586-1587.

Rivault, C. 1976. The role of the eyes and ocelli in the initiation of circadian activity rhythms in cockroaches. Physiol. Entomol. 1:277-286.

Roberts, S. K. de F. 1960. Circadian activity in cockroaches. I. The freerunning rhythm in steady-state. J. Comp. Physiol. 55:99-110.

1965. Photoreception and entrainment of cockroach activity rhythms. Science, Wash. 148:958-959.

Saunders, G. S. 1976. Insect Clocks. In: International Series in Pure and Applied Biology, Zoology Div., G. A. Kerkut, ed. vol. 54. Pergamon Press, New York.

SAS Institute, Inc. 1982. A. A. Ray, ed. SAS User's Guide: Basics. SAS Institute, Cary, NC.

Sombati, S. and G. Hoyle. 1984a. Generation of specific behaviors i a locust by local release into neuropile of the natural neuromodulator octopamine. J. Neurobiol. 15(6):455-480.

1984b. Central nervous sensitization and dishabituation of reflex action in an insect by the neuromodulator octopamine. J. Neurobiol. 15(6):481-506.

Sulzman, F., D. Ellman, C. Fuller, M. Moore-Ede, and G. Wasser. 1984. Neurospora circadian rhythms in space: a reexamination of the endogenous-exogenous question. Science 225(4658):232-234.

Tanaka, Y. 1950. Studies on hibernation with special reference to photoperiodicity and breeding of the Chinese Tussar silkworm. I. Seric. Sci. Japan 19:358-371.

Vancassel, M. 1968. Contribution a l'etude du role des yeux et des ocelles dans la prise et le maintien du rhythme d'activite locomotrice circadien chez Periplaneta americana. Ann. Epiphytes 19:159-164.

Wellington, W. G. 1974. Bumblebee ocelli and navigation at dusk. Science 183:550-551.

Wilson, M. 1978. The functional organization of locust ocelli. J. Comp. Physiol. 124:297-316.

Wolsky, A. 1930. Optische untersuchungen uber die Bedeutung und Funktion der Insektocellen. Z. Vergl. Physiol. 12:783-787.

1931. Weitere Beitrage zum Ocellenproblem - Die optischen Verhaltnisse der Ocellen der Honigbiene (Apis mellifera L.). Z. Vergl. Physiol. 14:385-389.

The vita has been removed from the scanned document