AN ABSTRACT OF THE THESIS OF

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Title: A STUDY OF SELECTED BEHAVIORAL AND ELECTROPHY-

SIOLOGICAL RESPONSES OF CARPENTER ANTS (CAMPAN-

OTUS) TO IONIZING RADIATIONS

Abstract approved: ___ Redacted for privacy ___
                          Donald J. Kimeldorf

Carpenter ants were found to exhibit immediate, transient be-

havioral responses to x-rays (300 kVp) at exposure rates ranging

from 0.05 R/sec to 80 R/sec. Within this range, the strength and

duration of responses, and the percent of subjects responding were

proportional to the exposure rate. The latency to response was in-

versely related to the exposure rate.

A comparison of behavioral reactions of normal subjects with

subjects whose eyes or antenna had been removed or shielded, re-

vealed that sensory receptors (probably olfactory) on the antennal

flagella were primarily responsible for detection and led to the onset

of prompt responses. The visual system was more importantly in-

volved in a transient cessation of activity (the "off" response) on

termination of exposure.

Bioelectric potentials were recorded from isolated eyes (electro-retinograms) and isolated antenna (electroantennograms) during brief exposures to beta radiations (^{90}Sr - ^{90}Y). The results largely corroborated evidence derived from behavioral studies that eyes and antennal receptors are sensitive detectors of ionizing radiation. The strength of bioelectric responses varied directly with stimulus strength. Estimates of threshold exposures or doses, delivered in fractions of a second, were: ERG response to beta radiation, 3.2 mrads; to x-rays, 30 mR; EAG response to beta radiation, approximately 200 mrads.

X-ray and visible-light stimuli elicited electroretinograms with very similar characteristics, suggesting a common mechanism of action on the photoreceptor pigment in the omatidia. The response to beta radiations displayed some unusual characteristics that cannot be explained at present.

Ants escaped from and avoided prolonged exposures to x-rays when tested in the fall of the year. Using ionizing radiation as an unconditioned, aversive stimulus it was possible to establish a strong conditioned avoidance response toward the place of exposure. However, exposures under identical conditions in the springtime resulted in movement of ants into, rather than away from, the radiation field. Attempts to condition a response toward the place of exposure failed. Other seasonal differences in responsiveness were also apparent.

It is suggested that ants exhibited a stimulus-seeking behavior in the spring, and a stimulus-avoiding behavior in the fall. These seasonal differences were shown to be unrelated to potential pheromone or to phototrophic effects. The evidence suggests that carpenter ants are physiologically different at different times of the year.

The motivational effects of ionizing radiation are apparently independent of mechanisms responsible for prompt detection, since ants exhibited an attraction to the beam during exposure in the spring even in the absence of antennal and visual receptors. Operated subjects were not studied in the fall of the year.

All prompt responses and motivational effects were reversible, and were exhibited with exposures from a few mR (bioelectric responses) to a few kR (motivational effects). By contrast, behavioral evidence of radiation-induced injury did not appear during continuous exposure until at least 82 kR had been delivered.

In lethality studies, the time of death, and the severity of behavioral symptoms of damage, were strongly dependent on total exposure. The LD 50/30$_{days}$ was 101 kR, and the LD 50/60$_{days}$ was 66 kR. Behavioral syndromes developing upon massive exposures indicated that injury and death resulted primarily from injury to the nervous system.

A Study of Selected Behavioral and Electrophysiological
Responses of Carpenter Ants (Campanotus) to
Ionizing Radiations

by

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Typed by Ilene Anderton for _ David Linnebach Martinsen_

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INTRODUCTION

The Nature of the Problem

Within a few months after the discovery of x-rays in 1895, various workers had reported that, under appropriate experimental conditions, x-rays could evoke visual sensations in man (see Lipetz, 1962), and could alter promptly the behavior of certain lower organisms (Axenfeld, 1897a).

Before the turn of the century it was learned that, while exposure to ionizing radiations could have deleterious effects on the tissues of organisms, x-rays could be used for theraputic purposes. Biological research in these areas received the attention of most investigators during the next several decades, with essentially no attention given to further study of the stimulus properties of ionizing radiation. Indeed, despite early evidence to the contrary, Schubert and Lapp reflected common opinion when they said in 1957 that

> Human beings are not equipped with any sense organs
> which respond to the presence of penetrating radiation.
> Man is essentially blind to the presence of radiation.

During the past 15 years or so there has been a resurgence of

interest in ionizing radiation as a stimulus, initiated primarily by

work done in the laboratory of D. J. Kimeldorf and colleagues, and

first reported in the mid-1950's (Kimeldorf, Garcia, and Hunt, 1955).

There now exists conclusive evidence that animals are capable of

detecting small amounts of ionizing radiation in their environment.

It is also known that penetrating radiation can act as a stimulus to

promote the learning of a conditioned avoidance responce toward dis-

tinctive cues associated with exposure. However, many intriguing

problems are yet to be solved in this relatively young field of investi-

gation.

Prompt Detection of Ionizing Radiation

The prompt detection, or "sensing", of stimuli of ionizing

radiation has recently been reported for some invertebrates (Hug,

1960; Smith and Kimeldorf, 1964; Kimeldorf and Fortner, 1971),

and for many species of mammals (see the reviews of Kimeldorf

and Hunt, 1965; Smith, 1971).

The procedure for demonstrating detection is relatively simple

for certain invertebrates, but more difficult for mammals. For

example, snails or sea urchins placed in a beam of x-rays quickly

retract their tentacles or tube feet. These simple, reflex-like be-

havioral responses illustrate that a stimulus is somehow detected by

the organism. By contrast, when an attentive mammal is simply

exposed to x-rays it does not typically change its behavior. The most

sensitive behavioral demonstrations of prompt detection have usually

involved the use of techniques developed in experimental psychology,

wherein the stimulus in question (ionizing radiation) is made to be-

come a conditional stimulus through prior association with a mildly

painful experience. For example, rats or monkeys, that have been

trained to press a lever for a food reward, will cease bar-pressing

behavior upon the intermittent delivery of a mild electric shock; they

do not stop pressing the lever when exposed to brief bursts of x-rays.

If, however, brief shock and x-ray stimuli are presented together

for several trials, each subsequent presentation of x-rays alone will

cause the subject to interrupt its bar-pressing behavior (Garcia,

et al. 1962; Taylor, et al. 1968). This is clear evidence that, under

appropriate conditions, mammals can detect the presence of ionizing

radiation in their environment.

Ionizing Radiation as an Aversive Stimulus

To explore the hypothesis that exposure to ionizing radiation

might be noxious or aversive to animals, experimental designs other

than those mentioned above have been necessary. One which has been

extremely useful in radiation studies is a modification of the class-

ical (Pavlovian) conditioning paradigm, called trace conditioning,

and is perhaps best explained by example. If rats are permitted to

taste a distinctive food cue (such as sweetened water) at or about the same time that they are exposed to radiation, they subsequently avoid this cue (refuse to drink sweetened water) for several days or weeks. (Control rats, given the same amount of radiation but not provided with sweet water until the next day, will subsequently drink 90 percent more sweet than distilled water). This suggests that ionizing radiation, in addition to being detected, can also be aversive, by somehow inducing the development of a negative motivational state in the animal. Stated differently, when ionizing radiation is briefly presented to a mammal as an unconditioned stimulus (US), together with some neutral but unique stimulus like sugar water (conditional stimulus, CS), the mammal associates the two stimuli and subsequently modifies its behavior so as to avoid the CS; that is, the CS becomes a conditioned aversive stimulus, providing evidence that the US was in fact aversive or unpleasant.

Rats, mice, cats, and monkeys have been conditioned to avoid various taste and location cues associated with exposure to ionizing radiation (see Kimeldorf and Hunt, 1965). However, no thorough investigation of motivational effectiveness of ionizing radiation has been done with any lower organism.

Mechanisms of Action of Ionizing Radiation

Most research attempting to explain how ionizing radiation acts

to produce prompt reactions has been done on mammals. Although the mechanisms of action are not well understood, it is known that exposure levels effective in eliciting behavioral responses are much lower than exposure levels necessary to cause significant tissue damage. Prompt behavioral evidence of detection typically occurs with exposure to only a few mR, and as little as 10 R has been found sufficient to effect a conditioned avoidance response to a taste cue in rats. By contrast, the exposure required to kill half of the irradiated subjects in most mammalian species within 30 days (LD $50/30_{days}$) amounts to several hundred roentgens. It requires in excess of 10,000 R to kill mammals within a few minutes or hours.

Routes of Detection

Mammalian studies have revealed that certain sensory systems are involved in prompt detection of ionizing radiation. Most approaches that have been employed to elucidate further the roles of specific sensory systems fall into two broad categories: (1) The system is either shielded from exposure or removed from the animal and the occurrence of a particular criterion of detection sought in the treated animal (Hunt and Kimeldorf, 1962; Taylor, et al. 1968). (2) Electrophysiological recordings are made to determine if exposure can evoke bio-potentials or alter the neural activity of the particular sensory system (Cooper, Kimeldorf, and McCorley, 1966;

Cooper, 1968).

Several generalizations have accrued from these studies: (1)
Immediate sensing is not demonstrably due to direct excitation of
nerves or muscles, despite the fact that high-energy radiation is
capable of simultaneously penetrating all tissues in the body. (2)
Visual and olfactory sensory receptors are intimately involved in the
immediate detection of ionizing radiation. (3) Detection is strongly
dependent on exposure rate rather than total exposure. Additionally,
threshold exposure rates, below which no detection is evidenced, are
remarkably similar (a few mR per second) for behavioral responses
and for electrophysiological responses of eyes and olfactory organs.

Aversive Effects

Despite several ingenious approaches to this problem, the
mechanisms by which ionizing radiation induces a negative motiva-
tional state in mammals are not known. Parabiont studies on rats
(Hunt, Carroll, and Kimeldorf, 1965; 1968) have revealed that a
humoral factor is involved in this species, but it is not known whether
this radiation-elicited factor is produced within, or simply carried
by, the blood.

It has been learned that the aversive response to ionizing radi-
ation is independent of mechanisms involved in prompt detection.
Rats that are deeply anesthetized during exposure in a trace

conditioning study will exhibit strong avoidance of the CS (saccharin solution) after exposure (Hunt and Kimeldorf, 1965). The same is true of mammals that have been surgically deprived of their main routes of detection, the eyes or the olfactory nerves (see Smith, 1971). Finally, the conditioned avoidance response is known to depend primarily on total, absorbed dose, in contrast to the dose rate dependence of radiation detection. That is, within limits, the strength of a conditioned avoidance response is independent of the rate at which ionizing radiation is delivered. Thus detection and aversive behavior appear to be separate and distinct reactions that occur as a result of exposure to ionizing radiation.

Justification for Using Insects as Subjects

Occasional reports of prompt, reflex-like responses to ionizing radiation have appeared for a few arthropods. Attempts to identify the mechanisms of detection underlying these prompt responses have implicated photoreceptors (Axenfeld, 1897a and b; Baylor and Smith, 1958; Smith and Kimeldorf, 1964; Terwilliger and Levy, 1964). Other sensory systems have not been studied, yet insect antenna are abundantly laden with a variety of sensory receptors and, as distinct appendages, are well suited to manipulative procedures and electrical recording. Additionally, the neural organization of insects results in predictable, stereotyped behavioral responses to

environmental stimuli such as ionizing radiation. In mammals, the
demonstration of detection is generally much more difficult because
of the complexity of neural organization and compensatory mechan-
isms that tend to buffer the effects of stimulation. If behavior is to
be used as an end point, elaborate conditioning techniques are usually
required wherein ionizing radiation is made to become the conditioned
stimulus.

Specifically, ants were chosen for this study because: (1) Mem-
bers of some species have been reported to respond immediately
(within four seconds) upon exposure to x-rays (Hug, 1960), yet noth-
ing is known about mechanisms of detection. (2) Scattered reports
suggest that ionizing radiation might lead to aversive behavior. Radi-
ation exposures have reportedly resulted in aggressive behavior and
rapid escape from the beam for carpenter ants (Hug, 1960). A
gradual emigration of Formica integra from a natural habitat near
the field exposure site of Brookhaven National Laboratory has been
observed (Brower, 1966). Krebs and Benson (1966) found that high
intensity radiation fields interfered with normal patterns of digging
behavior in the ant, Pogonomyrmex californicus.

No further work has been done on motivational effects with any
arthropods, let alone ants. It should be of interest to learn whether
or not ionizing radiation is in fact aversive to animals other than
mammals, since comparative studies might lead to a better

understanding of mechanisms responsible.

Specific Objectives of This Study

Using carpenter ants (Campanotus sp) as subjects, I have attempted to (1) identify several prompt behavioral and electrophysiological responses to brief bursts of ionizing radiation, (2) characterize these reactions as a function of the physical factors of exposure, (3) illuminate certain of the mechanisms by which ionizing radiation acts to alter immediate and long-term behavior, (4) describe the motivational effects of ionizing radiation on behavior, and (5) define the lethality response of carpenter ants to ionizing radiation.

METHODS AND PROCEDURES

The Experimental Animal

Classification

Ants used in this study belong to the genus Campanotus, members of which are found throughout the temperate and tropical zones of the world. According to Wheeler and Wheeler (1963), Campanotus is the largest genus of ants, comprising some six hundred species which are grouped into forty subgenera; in the Nearctic fauna, there are thirty nine species in eight subgenera. Most species in this genus build their nests in decaying wood, a habit resulting in their common name, "carpenter ants".

The two principle subjects of my investigation are members of the subgenus Campanotus. Both forms have been considered varieties of the same species, C. herculeanus modoc and C. herculeanus laevigatus (Smith, 1947). Other investigators (Creighton, 1950) refer to them as separate species, C. herculeanus (the northern or modoc carpenter ant) and C. laevigatus (the giant carpenter ant). There is a great deal of disagreement among myrmecologists regarding classification and species distribution of the entire genus Campanotus (Creighton, 1950). Since Creighton's work is the most recent and authoritative treatment of this subject, I shall refer to northern and

giant carpenter ants as separate species.

Both species are about the same size, the body of worker ants ranging between five and 15 mm in length (Figure 1). The body of "northerns" is a dull black, whereas the petiole, legs, and antennal flagella are a deep red. Yellowish abdominal hairs are relatively short and reduced. In the United States, this species is found throughout the northern states, with southern extensions in mountain ranges of the east and west. By contrast, the entire body of the "giants" is shiny, jet black, and the abdomen is covered with dense, erect, whitish hairs. Its distribution is restricted to the Pacific Coast States, eastward to the Rocky Mountains.

Life History

Highlights of the development and functioning of a colony of C. herculeanus in a temperate climate is presented below, based on information from Schneirla (1951) and Wheeler (1910), and on my own experience in the field.

Carpenter ants undergo a complete metamorphosis, developing from egg to larva to pupa to adult. A mature colony contains several thousand members with a variety of forms and functions. Individuals to which one commonly applies the name "ant" comprise the worker caste, which functions primarily in fighting, foraging, nursing the developmental stages, and general maintenance of the colony. A
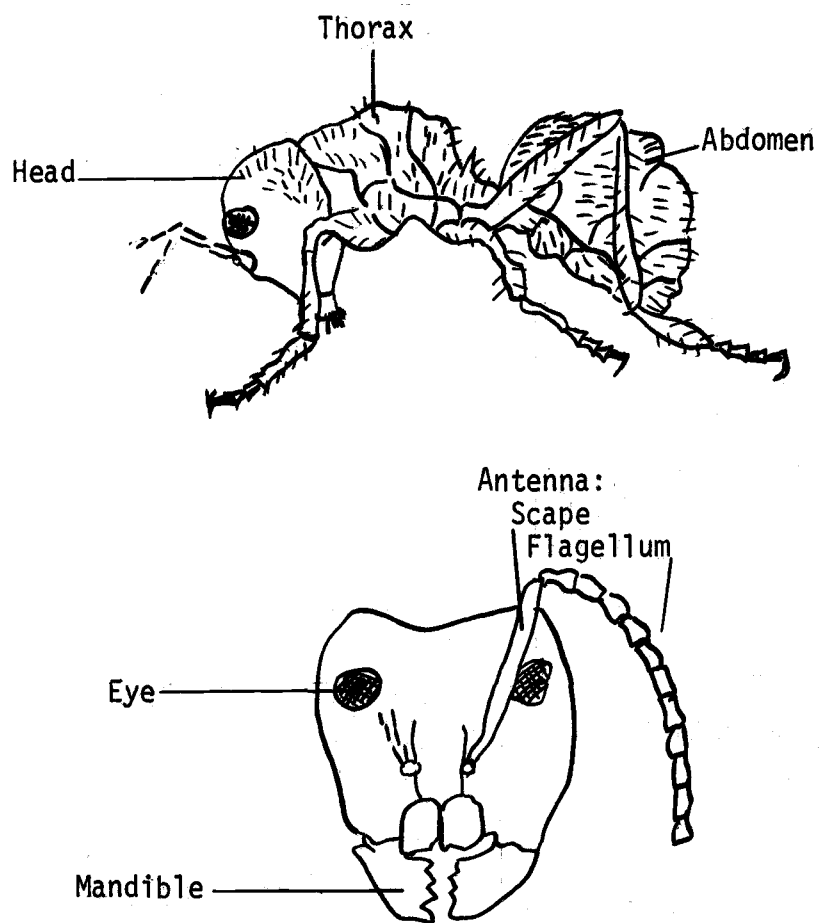
Figure 1. Enlarged sketch of a worker carpenter ant, showing
major regions of body (above), and the head.

worker differs from the female (queen) in that its reproductive organs are nonfunctional, it is smaller, and lacks wings and ocelli (simple eyes). Workers can be divided, according to size, and, to some extent function, into minor workers, intermediate workers, and major workers. In addition to workers, a mature colony consists of larva and pupa in varying stages of development, one or more queens, and many winged males and winged females.

Certain species of carpenter ants, including those employed in this study, are unique among ants (Wilson, 1963) in that the entire colony enters into true diapause in the late fall. Mating flights (swarming) occur shortly after the colony resumes activity in the spring, between March and June. The winged males and winged females that participate in swarming may develop from eggs laid by the queen at any time, but as a general rule they mature into adults in the summer, overwinter in the nest, and swarm in mating flights shortly after emerging in the spring. The mating flights of colonies in the same area occur simultaneously, affording a means of interbreeding.

After mating, males die and newly-fertilized queens attempt to establish new nests in the cracks or crevices of stumps, old logs, and the like. Several eggs are laid by a new queen, which subsequently form the nucleus of a new colony. The few workers that emerge from the first pupa are small, minor workers, but

immediately assume all duties of the colony. They open the chamber to the outside, collect food, begin to excavate galleries to enlarge the nest, and tend eggs, larvae, and pupae of the second generation. These workers regurgitate food for the developing larvae and for the queen, who hereafter ceases all overt activity except egg laying.

In subsequent generations, workers of various sizes are produced. Each individual worker tends to specialize in one or two duties of the colony. In general, larger ones (major workers, sometimes called soldiers) guard the nest against intruders, explore, and forage for food. Smaller workers are commonly occupied with expanding the nest and caring for the young. These duties overlap, and usually some members of each caste are engaged in all occupations.

Collection and Maintenance

Both C. herculeanus and C. laevigatus were collected from the same site, a marshy area near Corvallis, Oregon, in which there were several old fir trees in varying stages of decay (Figure 2). Northerns and giants were often found, in separate colonies, within the same log.

Two methods of collection were employed. Ants could be gathered up individually during their activities outside the nest galleries (Figure 3) from March or April until October, and maintained in small groups within Turtox Ant Nests for short periods prior to

Figure 2. Old logs at the collection site of all ants used in this study.

Figure 3. Several workers of <u>C</u>. <u>herculeanus</u> on one of the logs seen in Figure 2.

experimentation. During the last year of the study, I used a chain saw to remove, from a large log, a section of the nest containing several hundred ants. This was placed in a 10-gallon terrarium and transported to the laboratory, from which ants were then transferred directly, as needed, into experimental chambers.

Ants were maintained in laboratory colonies at room temperature, on approximately a 12/12 photoperiod, for at least several days (and as long as four months) prior to experimentation. Water was provided via moistened cotton pads, and food consisted of bits of insect bodies and grains of sugar.

It was necessary to maintain each species in separate containers. Under natural conditions, encounters between giants and northerns usually resulted in avoidance behavior. However, if placed together in the confinement of a small container or nest, ants of both species (or of the same species from different colonies) became highly excited, exhibiting aggressive behavior toward each other, which usually resulted in the death of one or both ants.

Partial colonies of ants maintained in the laboratory often contained members of all castes, and some developmental stages. Most ants, regardless of caste, gave similar responses to a variety of stimuli, including ionizing radiation. However, to minimize one potential source of variation, the work reported herein was done on

major worker ants of approximately the same size (10 - 12 mm in length). Additionally, the behavioral responses of both species were remarkably similar, but the results reported in this thesis, with one notable exception (to be discussed) are from experiments done with C. herculeanus.

Studies of Prompt Detection

Methods for the Study of Behavior

Identification of Prompt Behavioral Responses

I have defined prompt, reflex-like responses to be behavioral reactions that were elicited within seconds of the onset of exposure and were transient. Some readily-identifiable and consistent prompt responses included head bobbing, brisk waving of antenna, and a startle response involving an abrupt rapid running behavior. Prompt responses ceased either during, or soon after, termination of the stimulus. Two main prompt responses were chosen for careful analysis: (1) antennal waving, and (2) rapid running behavior.

The latency of a response was the duration of time from the onset of exposure to the start of a specific response. The strength or intensity of the running response was assumed to be reflected by the speed of running, which was determined by measuring the distance traveled per unit time (cm/sec or cm/min). Other characteristics

of prompt responses that were expressed quantitatively were the percent subjects responding, and the duration of a response.

Observation Methods

Remote observation of ants during exposure to x-ray stimuli was made by a closed-circuit television system. A Shibaden Model HV-15 camera, equipped with an f 1.4, 25mm lens, was positioned and focused close to the exposure chamber in the x-ray room. A TV monitor and videotape deck (Apeco Model VT 101) were on line and set up in the x-ray control room.

Videotape records were made of most exposures. An audio-channel of the tape deck allowed the recording of a tone emitted by the x-ray generator that corresponded to the precise period of exposure. The audiochannel also allowed insertion of appropriate comments during an experiment. Then tapes were replayed in the laboratory as many times as desired, for the extraction of data on the latency, strength, etc. of the behavioral response in question.

Exposure Methods

X-Rays. The x-ray source was a General Electric Maxitron 300 therapy unit. The port of the x-ray machine was positioned directly above an ant exposure chamber. This exposure chamber, in turn, rested atop 15 cm of high-density rubber pads in order to

maximize backscatter.

Except for one experiment (below), all exposures were made at 300 kV and 20 mA, with the inherent filtration of the x-ray tube equivalent to 0.25 mm aluminum. Under these conditions, with no external filtration, the exposure rate could be varied from 0.7 R/sec to 80 R/sec by altering the distance between the tube target and the exposure chamber.

To provide exposure rates for threshold determinations of prompt responses it was necessary to either (1) increase the beam filtration by thin absorbers, thereby "hardening" the beam by removing a greater proportion of low-energy x-rays, or (2) decrease the maximum kilovoltage applied to the tube, thus "softening" the beam by eliminating the high-energy component. These variations in beam quality were employed only in the determination of exposure rates below which no prompt responses were elicited.

Exposure rates were measured, and frequently rechecked, with a calibrated Victoreen R-meter and a matching set of thimble chambers. To measure an exposure rate in air, a thimble was inserted into the center of an ant chamber and exposed under experimental conditions. The distance between the x-ray tube target and the chamber was then adjusted to yield the desired exposure rate. Exposure chambers for ants were designed to be small enough, relative to the exposure field, that the measured variation in rate throughout a

chamber was usually not greater than the variation among three read-

ings taken at the center of the chamber.

Exposure chambers were single-compartment, 30-cc plastic

tissue culture flasks (Falcon Plastics), 2 cm X 3.5 cm X 8 cm

(Figure 4). Small holes drilled in the top provided ventilation and

permitted easy introduction of food and water. Experimental subjects

were transfered from the main laboratory colony to the chambers be-

tween 24 and 30 hours prior to exposure.

Preliminary tests revealed no differences in the latency or

strength of prompt responses between ants exposed individually (one

ant per chamber), and those exposed in small groups. Thus, unless

mentioned otherwise, ants were maintained and subjected to test

stimuli in groups of five to eight ants per chamber.

Most exposures to x-rays were of 20-30 seconds duration, al-

though tests were made with exposures lasting as little as two seconds,

and as long as five minutes.

To minimize the risk that spontaneous changes in behavior

would be interpreted as radiation-induced, experimental subjects

were required to achieve and maintain a resting posture for at least

one minute prior to exposure. Each experiment was recorded on

tape for one minute prior to exposure, the duration of exposure, and

one or more minutes post exposure.

Except in one experiment, which was designed to test the
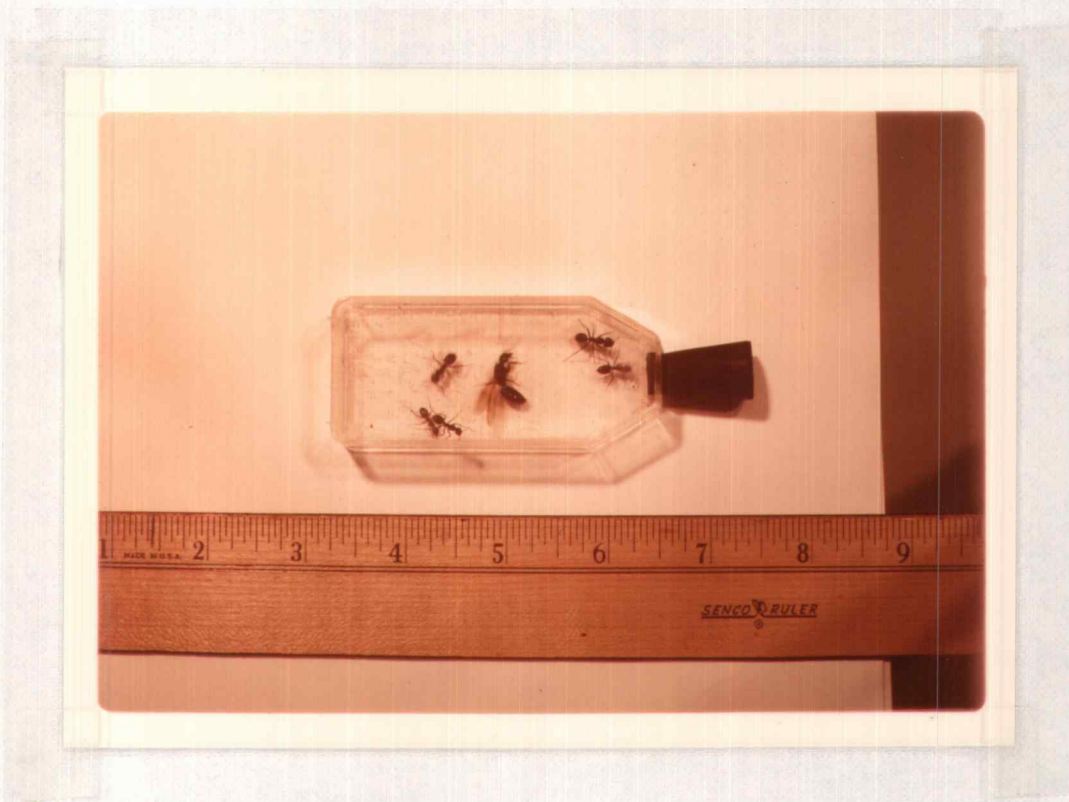
Figure 4.   Northern carpenter ants in a single-
compartment exposure chamber.

effects of repeated exposure on prompt behavioral responses, an ant

or group of ants was exposed to radiation only once, then discarded.

Unless otherwise mentioned, exposures were made in a well-lighted

room, to mask any potential effects of x-ray-induced fluorescence

in the walls of the exposure chamber.

Tests were made to eliminate the possibility that noise associ-

ated with the operation of the x-ray machine might be acting as a

(contaminating) stimulus, by placing a lead shield over the chamber

prior to turning on the beam. In this manner subjects were exposed

to machine noise and to other potential extraneous stimuli associated

with irradiation, but not to x-rays. No responses were seen under

these conditions.

Electric Shock. It was sometimes necessary to subject experi-

mental animals to known stimuli other than ionizing radiation, either

for comparative purposes or to insure that surgically manipulated

subjects were capable of making appropriate responses. To provide

an electric shock, I placed narrow, parallel strips of aluminum foil

in the false bottom of an exposure chamber, and alternately connected

these strips to the opposite poles of a D. C. power supply. Voltage

could be varied from 1 to 20 volts. A subject was placed on the in-

activated grid and allowed to settle such that its body bridged two

adjacent strips of aluminum and would thus short circuit the system.

Then the power was turned on and the voltage gradually increased

until I observed a response. Alternatively, the voltage was set high

enough to cause an immediate response from a normal ant, then

turned on. In this way the latency to react, and the strength of the

response, could be studied.

Light. The behavioral responses of dark-adapted ants to the

stimulus of visible radiations were tested by exposure to a 200-watt

incandescent bulb source. The light was positioned above an exposure

chamber and observations were made in a room dimly illuminated

with a 15-watt red light. Exposures were controlled by a hand set

switch.

Odors. Several odors were employed to test the behavioral re-

sponse of normal and experimental subjects to olfactory stimulation.

The strongest and most predictable responses (immediate attack

behavior) were elicited by the natural odor of a foreign ant (an ant of

a different species or from a different colony). This stimulus was

presented with equal effectiveness by either (1) introducing a freshly-

killed foreign ant, impailed on a toothpick, into the test subjects'

chamber, or (2) introducing the odor by first crushing the body of the

ant on filter paper, then inserting the paper into the chamber. In

either case, the odors of nestmates were introduced as controls, to

insure that visual or mechanical cues were not involved in the re-

sponses observed.

Surgical Alterations. Both partial and complete antennectomy

and ophthalmectomy were employed to assess the role of antennal and visual receptors in the detection of ionizing radiation. All operations were performed under a binocular microscope on ants which had been lightly anesthetized with carbon dioxide. Control subjects for each experiment were treated exactly as experimental subjects except for the amputation (sham operation procedure). After perfection of surgical technique, the mortality of experimental animals was no greater than that of anesthetized controls (0-20 percent). In all cases, a post-operative recovery period of at least 24 hours was allowed prior to experimentation.

One or both antennae (or parts thereof) were removed by cutting between two adjoining segments with iridectomy scissors. Care was taken to cut precisely at the intersegmental joint, thereby preventing excessive loss of body fluid, and facilitating quick recovery.

To insulate the intact antenna from odors in one study, purified white shellac was applied to the antennal flagella with the aid of a fine-tipped brush. Shellac was nontoxic, dried quickly, and formed a seamless coat over the flagella.

For ophthalmectomy, the corneal surface of each eye was initially pierced with the sharpened, sterilized tip of an insect pin. Then a fine watchmakers forceps was employed to remove the cuticle, corneal lens, and underlying crystalline structures containing the receptor cells. It was not possible to be sure through visual

inspection that all photoreceptive cells in the compound eye had been removed or destroyed, but operated subjects did not, after dark-adaptation, respond to bright flashes of light, as did sham-operated controls.

Electrophysiological Methods

Visual Mechanisms

A Description of the Electroretinographic Response (ERG). When a suitable stimulus is flashed onto the compound arthropod eye, a potential can be measured between the eye and a distant reference point. This electroretinographic response of the eye is apparently a reflection of the bioelectrical shifts that occur with depolarization of many receptor cells and underlying neurons. The process occurs rapidly and is initiated by the absorption of photons by visual pigment molecules within the receptor cells. Considerable variation in this response exists among the eyes of different arthropods (Bullock and Horridge, 1965), but certain features are relatively predictable and were chosen for analysis in the present study. (1) The initial, corneal-negative potential, or "on" response, develops within milliseconds of the onset of exposure, then declines rapidly to zero. The termination of the stimulus causes the appearance of a potential of opposite polarity, the "off" response. Together these constitute the

ERG. (2) The ERG does not develop if the intensity of the stimulus changes slowly, even though the same absolute change in intensity occurs. (3) A flash of light reduces the sensitivity of the eye to subsequent flashes. (4) Within limits, the strength of the ERG is proportional to the strength (intensity) of the stimulus.

Technique of Preparation and Recording. Initial recordings were made from the eyes of intact ants immobilized in modeling clay. To avoid intermittent interference by large, D. C. muscle potentials, subsequent preparations involved severing the head from the body, removing the antenna and mouthparts, and then partially imbedding the head in clay or wax.

With the aid of micromanipulators and a binocular microscope, a Transidyne No. 410 platinum-clad microelectrode was inserted into the cuticle of the eye to a depth of about 0.2 mm. A Grass E 2 platinum reference electrode was positioned in the base of the head. A stereotaxic device held the eye clamp in place, and the entire preparation was mounted on a solid plywood base. The essential components of this set up are illustrated in Figure 5. Lucite components of the eye holder were painted flat black, to prevent any potential radiation-induced fluorescence from stimulating the eye.

No difference was apparent between the electroretinographic recordings of isolated and intact eyes. This finding has been true in the studies of other arthropods as well (see Hartline, 1928).
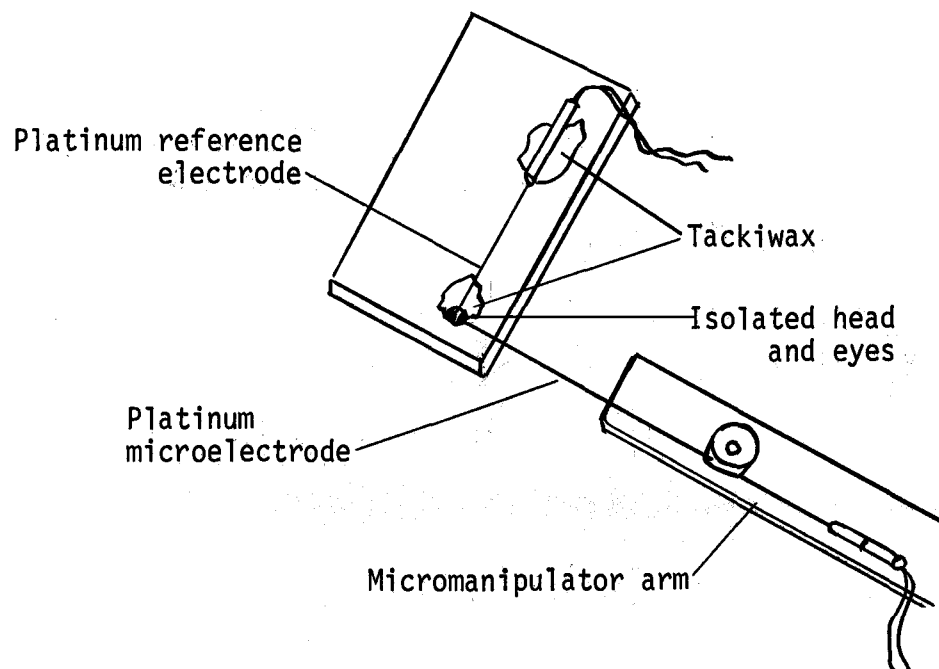
Figure 5. Essential components of the isolated eye
preparation. Approximately actual size.

Repeated tests showed that the responsiveness of the isolated ant eye

did not begin to decrease for at least three hours after initial records.

All results reported here were obtained from fresh preparations.

Electroretinograms were recorded on a Grass Model 7 poly-

graph, in conjunction with a Model 7P1-A low-level D. C. preampli-

fier, and a Model 8P-A ink-writing oscillograph.

Exposure Technique. I exposed the eyes of ants to stimuli of

(1) light, (2) beta radiation, and (3) x-rays. The exposure apparatus

and dosimetry for each stimulus modality will be described below.

The typical procedure used to study the ERG was as follows: each

eye was dark-adapted for several minutes, then stimulated by flashes

of light for standardization of response, followed by test stimuli of

ionizing radiation. The physiological integrity of the eye preparation

was tested at intervals by checking the response to visible light

stimuli. In this way, comparisons could be made between responses

given to ionizing and non-ionizing radiation, using each eye as its

own control.

(1) Light--The isolated eye preparation was enclosed in a light-

tight box and allowed to dark adapt. Light, originating from a Bausch

and Lomb 150-watt xenon lamp, was passed through a visible mono-

chronomator grating (350-800 nm) and flashed onto the eye through

a small opening in the box. Flash duration was controlled by a leaf-

type camera shutter fixed to the exit slit of the monochromator.

Flash intensity was varied by changing the distance between the light source and the eye or, at a fixed distance, by attenuating an initial intensity to 0.50, 0.25, or 0.01 with Ealing-TFP neutral-density filters. Intensities were measured in ergs/cm^2-sec with a YSI Model 65 radiometer.

During preliminary work, one eye was stimulated with specific wave-lengths from 350-800 nm. Appropriate band-pass and cut-off filters were used to reduce overlapping wavelengths. An action spectrum derived from the results showed peaks at 360 and at 500 nm. Further testing showed that the eye responded with maximum efficiency at 500 nm, thus subsequent experiments, designed to compare the effects of visible light and ionizing radiation on the eye, were done using this wavelength of light.

(2) Beta Radiation--The principle source of beta radiation was a 100 mCi sealed, Tracerlab Medical Applicator, containing strontium-90 and yttrium-90 in equilibrium. Maximum energies (E_{max}) of the near-pure beta radiation emitted by these isotopes were 0.54 and 2.25 Mev, respectively. The applicator cup containing the source was approximately 1/2 inch X 1/2 inch, capped with a very thin steel window, and mounted on a seven inch shaft. The shaft was equipped with an adjustable plexiglass shield, for protection while handling.

A 50 mCi source of the same dimensions was used as well to determine the threshold dose rate for eliciting the ERG. Dose rate

measurements were made by means of lithium fluoride thermolumi-

nescent dosimetry (Jordan, 1970). Small calibrated LiF dosimeters

were positioned exactly where the eye was to be exposed, and the

beta exposure apparatus (described below) delivered short bursts

(flashes) of radiation. Table 1 lists the conditions of exposure and

the resultant values obtained from the dosimetry measurements.

Table 1. Absorbed Dose of Beta Radiation Measured at the Eye.

Exposure rate	Exposure duration (sec)	Dose per flash (mrads)	
		100 mCi	50 mCi
1/sec	1/20	11.6	3.1
2/sec	1/40	5.8	1.6
3/sec	1/60	3.5	0.9

For exposing the eye to beta radiation, the outer margin of a

lead-lined, steel disc was positioned between the beta source and

the eye preparation. The disc had two 5/16 inch holes drilled in its

margin, 180 degrees apart, and was aligned such that rotation of the

disc with a precision, variable-speed D. C. motor exposed the eye

to two flashes of radiation per revolution. As one hole exposed the

eye to the stimulus, the opposite hole exposed a photocell to a flash

of light from a shielded light source. Output from this photocell led

into one channel of the oscillograph, serving as a stimulus monitor.

At its slowest speed of rotation, this apparatus allowed for

presentation of stimuli at about 1 flash per second, with each flash being 1/20 second long (Table 1). Increasing the speed of rotation increased the stimulus rate and decreased stimulus duration, but for any given interval, say one minute, the net exposure time (thus total dose) remained constant. Smith and Kimeldorf (1964) designed this exposure apparatus, and it is illustrated and thoroughly explained in their paper.

To study a beta-induced ERG, room lights were dimmed for dark adaptation, then a train of beta stimuli presented to the eye. Considerable variation in responsiveness to beta stimulation existed among the eye preparations. To obtain a comparison of the relative responses of a given eye to beta radiation and to visible light, light stimuli were presented before, during, and following the delivery of beta stimuli.

(3) X-Ray Stimuli--X-ray exposures were made with a General Electric Model D 2 diagnostic x-ray machine, operated at 70 kV with a tube current of 1-10 mA, and a measured HVL of 0.58 mm aluminum. The x-ray tube head was mounted on top of a lead-lined cabinet, which served as the exposure chamber. The eye preparation (Figure 5), mounted on a sturdy base, could be inserted into or removed from the cabinet, as desired, without disturbing the implanted electrodes. This made it possible to alternately stimulate an eye with visible light and x-rays.

X-ray exposures were made by a solenoid-driven focal plane lead shutter that was manually controlled (Figure 6). In contrast to the beta exposure apparatus, the stimulus duration and interval between stimuli could be varied independently. However, the limitations imposed by the relatively high-inertia lead shutter made it impossible to attain stimulus rates and durations of greater than four per sec or less than 1/10 sec, respectively.

The onset and duration of each x-ray stimulus was monitored by a photocell covered with a fluorescent screen and suspended in the exposure chamber directly beneath the eye preparation (Figure 6). The photocell was enclosed in a black cup to prevent the escape of visible light, and its output was fed into one channel of the recording oscillograph.

Exposure rates were varied by changing the tube current. A victoreen R-meter and a 25 R thimble chamber were used to measure exposure rates in air. The thimble chamber was positioned at the site where the eye was to stimulated. Three exposures at each tube current setting were averaged and expressed as R/sec (Table 2).

X-ray tube

X-ray beam path

Lead shutter   Solenoid

Opaque plastic cap

Fluorescent screen

Photocell

Isolated eye
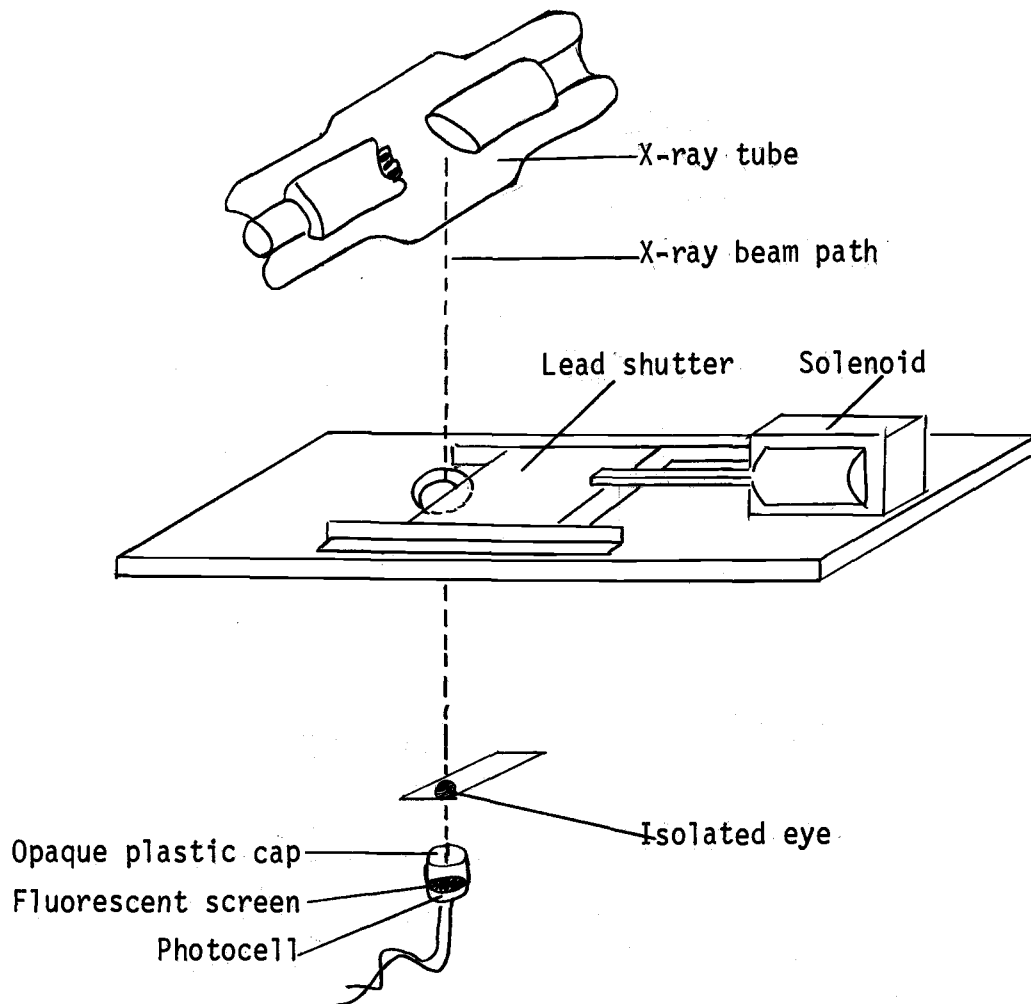
Figure 6.   Essential components of x-ray exposure apparatus
            for ERG studies.

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Table 2. Exposure Rates used in Stimulating the Eye with X-rays
From the Diagnostic Unit.

Tube current (mA)	Exposure rate (R/second)
1	0.3
3	0.8
4	1.2
5	1.5
6	1.8
8	2.3
10	2.7

Antennal Mechanisms

A Description of the Electroantennographic Response (EAG).

The bioelectric response of the insect antenna, named th electroanten-

nogram by Schneider (1957a), has been less well studied than the ERG.

When an adequate olfactory stimulus impinges on the insect antenna,

a relatively slow electrical potential difference develops between the

tip and the base. This potential is thought to reflect the summed re-

ceptor potentials of many olfactory sensory neurons (Schneider, 1963).

The precise form of the EAG depends on the odor stimulus and

on other factors which are not understood. Some generalizations are

apparent. Within milliseconds after the onset of a stimulus, the api-

cal region (tip) becomes negative relative to the base, and may remain

depolarized, or return to zero, during stimulation. An "off" response or a reversal in polarity is uncommon. An odor stimulus reduces the sensitivity of the receptors to subsequent stimuli. Recovery can be rapid (msec) or slow (min), depending on the quality and quantity of the stimulus. The magnitude of the EAG change is proportional to the strength (concentration) of the odor stimulus.

Technique of Preparation and Recording. The antenna had to be completely isolated from the rest of the body for proper recording of the EAG because odors, used as stimuli, often evoked potentials associated with muscular movements of the mandibles or of the scapus muscles joining the antenna to the head. The isolated antenna was mounted with tiny, insulated brads atop the flat surface of a cork. The cork mount was secured to a weighted ringstand by a clamp providing three-way position adjustments. Two Grass E 2 platinum electrodes were affixed in separate micromanipulators. The tip of one electrode was then manipulated into the base of the antennal scape. The other electrode was secured within a fine-tipped glass pipette filled with 1.0 percent saline, into which the distal segment of the flagellum was inserted (Figure 7).

Electrical recording was accomplished with a Grass Model 7 polygraph, in conjunction with a Model 7P1-A low-level D. C. preamplifier, and a Model 8P-A ink-writing oscillograph.

Exposure Technique. The isolated antenna was stimulated with
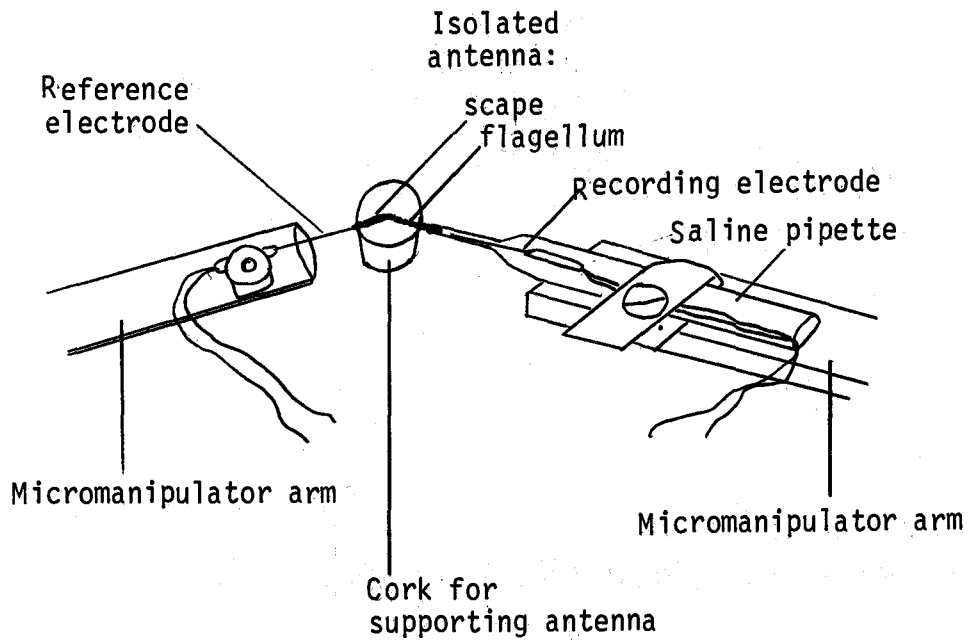
Figure 7.   Isolated antenna preparation for recording
            electroantennograms. Approximately actual size
            except for antenna and tip of saline pipette,
            which are enlarged to show detail.

a variety of odors which had proven effective in eliciting behavioral

responses. After perfecting techniques for recording the EAG, beta

radiation was used as a stimulus. X-ray exposures were not at-

tempted since the diagnostic x-ray machine which provided high-

energy photons for the ERG studies was not available for antennal

work.

All results were obtained within 30 minutes of isolating the

antenna, after which dehydration usually resulted in diminished re-

sponsiveness.

To administer various odor stimuli, a system was devised which

provided a constant flow of air over the isolated antenna, into which

a test odor could be injected periodically. One end of a length of ty-

gon tubing (0.8 cm dia.) was connected to a Silent Giant aquarium

air pump; the other end of the tube was mounted on the adjustable

arm of a micromanipulator and positioned directly above the isolated

antenna preparation seen in Figure 7. When the pump was turned on,

the current of room air striking the antenna was varied by adjusting

the manipulator arm up or down. To prevent excessive drying of an

antenna while it was not being stimulated, a 3-way valve, inserted

into the air line, allowed part or all of the flow to be shunted from

the preparation.

To present a train of odor stimuli (such as the odor of formic

acid), the air flow was diverted onto the antenna, causing a sharp

voltage signal, which was presumedly due to air striking the antennal mechanoreceptors. This deflection quickly returned to baseline. The tip of a clean glass rod was dipped subsequently into the test solution and passed back and forth through the flow of air impinging on the antenna. Control tests were made in the same way, using distilled water rather than an odorous solution. The approximate initiation and duration of each stimulus was manually recorded on a separate channel of the oscillograph.

Beta stimuli were presented manually, with the 50 mCi Tracerlab Medical Applicator described above. The air jet (above) was removed, and a lead shield was placed over the antenna, with a small window located directly above and one centimeter out from the antenna. To deliver a train of stimuli the beta source was passed back and forth over the window. Speed of movement of the source determined the stimulus duration, whereas intensity was varied by altering the distance between source and antenna. Table 3 lists the approximate dose rates employed.

The values for dose rate in Table 3 were calculated from a calibration value of 3.2 rads/sec at 1 cm, and from measurements of dose rates at various distances from the 100 mCi source (Jordan, 1970).

Each exposure to beta radiation was preceded and followed by a sham exposure with a Tracerlab non-radioactive applicator. These

sham exposures were made to detect any potential artifacts that

could be induced by the manipulation of the radiation source close to

the antenna.

Table 3. Dose Rates of Beta Radiation at the Position of the
Isolated Antenna.

Dose rate (rads/sec)	Distance from the 50 mCi source (cm)
3.20	1
2.20	2
0.37	4
0.15	6

My major objective was to determine whether antennal recep-

tors would give measurable bioelectric responses to ionizing radia-

tion. Other tests were made to (1) find out the effect of one stimulus

on responsiveness to subsequent stimuli of the same or different

modalities, and (2) establish the threshold dose rate of beta radiation

necessary to elicit an EAG.

Radiation as an Aversive Stimulus for Behavior

Criteria for Avoidance Behavior

For this phase of the research I selected two behavioral end

points which, if observed, would constitute evidence that

x-irradiation was an aversive stimulus for carpenter ants: (1) escape from, and avoidance of, the beam during exposure, and (2) the development of a conditioned avoidance response toward the place of exposure.

Exposure Methods

Several two-compartment, free-choice exposure chambers were constructed from 1 mm plexiglass, with dimensions of 2 cm X 2 cm X 15 cm. Each chamber was divided into two equal compartments by a lead partition; free access to both compartments was possible through a small hole in the partition (Figure 8). This design allowed one compartment to be exposed while the other was shielded with lead.

A strip of black tape was placed along the top of each chamber to equalize lighting conditions within the chamber. In some experiments, it was desirable to make one compartment discriminative. This was accomplished in two different ways. In the standard chamber, used for most experiments, the floor and walls of one compartment were thoroughly roughened, while those in the other remained smooth. A variation of this design, intended to enhance further the stimulus difference between the two compartments, was used for one experiment. It consisted of filling the rough-floored compartment loosely with assorted pieces of plexiglass "furniture", and painting the bottom of that compartment black.
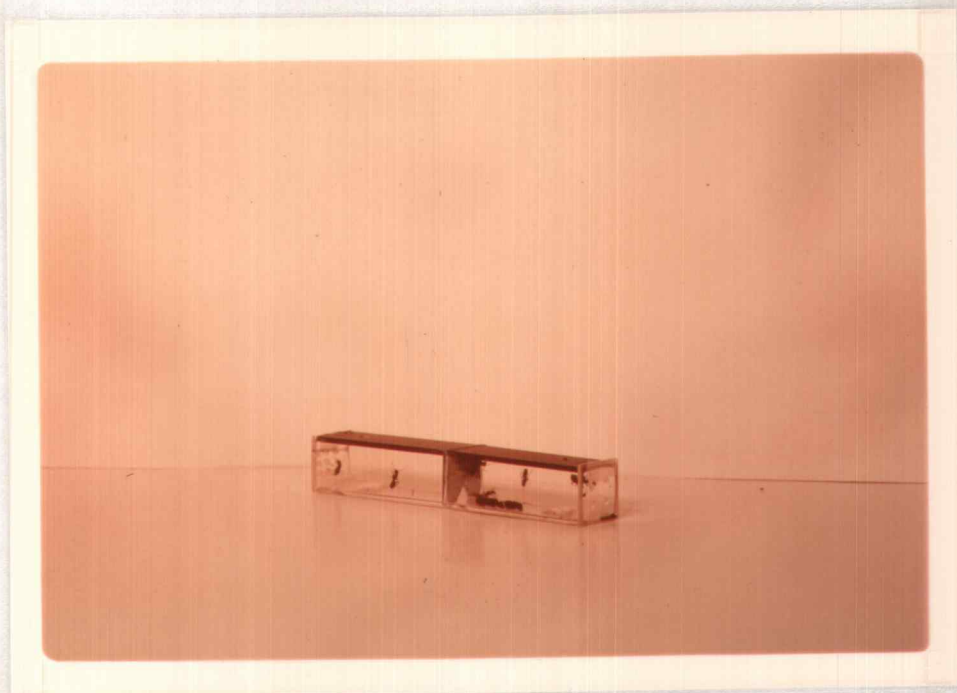
Figure 8. Northern carpenter ants in a free-choice exposure chamber.

The number of ants placed in a free-choice chamber varied from one to 14, depending on the experiment.

The x-ray source was the General Electric Maxitron 300 therapy unit described above. Except for the fact that this phase of the study utilized two-compartment chambers, conditions of exposure and dosimetric measurements were the same as described in the section on Studies of Prompt Detection. Eight millimeters of lead sheet placed atop one compartment of a free-choice chamber reduced the exposure rate within the shielded compartment by two orders of magnitude.

All exposures were made at 300 kVp and 20 mA, with the inherent filtration of the x-ray tube equivalent to 0.25 mm aluminum. Exposure rates employed to study radiation as an aversive stimulus ranged from 1 R/sec to 80 R/sec. Exposure duration ranged from one minute to over 100 minutes, depending on the particular experiment.

Observation and Recording Methods

Videotape records were made of each experiment for a period prior to, during, and following exposure to x-rays. The technique of recording was very similar to that described for recording prompt behavioral responses. To score the responses of escape and avoidance during exposure to x-rays, videotape records were replayed in

the laboratory. The percent or fraction of the exposure period spent in the unshielded compartment was recorded for each experimental subject.

Investigations of conditioned place avoidance required a more elaborate protocol. A three-day habituation period was allowed between the introduction of ants into a free-choice chamber and their exposure to x-rays. During this period, and for several days following exposure to x-rays, the behavior and distribution of ants within the chamber was studied. It was not feasible to observe and record behavior for continuous periods of time in excess of one hour. To obtain a quantitative estimate of the daily distribution of ants between the two compartments of a free-choice chamber, I scheduled several (8-20) observation points at intervals throughout the day. Each observation point consisted of recording the proportion of ants residing in the rough-bottomed compartment. The proportion was averaged for all observation points during the day to obtain a preference score for the rough-bottomed compartment.

During the habituation period, after initial exploration the ants in most groups exhibited a strong preference for the rough-floored compartment. Thus, this compartment was designated for exposure to x-rays, whereas the smooth-bottomed compartment was shielded with lead.

In this conditioning design, x-rays were the unconditioned

stimulus (US), and cues associated with the unshielded compartment, such as the rough floor, the conditional stimulus (CS). Assuming that ants would be capable of learning a simple problem, I reasoned that an x-ray stimulus, if noxious to ants, would provoke ants to escape into the shielded compartment. This act would effectively remove both the noxious US and the CS. Although the CS was initially preferred (described above), a sufficient number of pairings of the US (x-rays) and the CS (residence in the rough-floored compartment) should result in a conditioned avoidance response toward the rough-floored compartment, even in the absence of x-rays.

A typical experimental design consisted of three phases: (1) pre-exposure habituation phase, (2) exposure conditioning phase, and (3) post-exposure test of conditioning. A brief description of each phase is given below.

Pre-exposure habituation phase - several ants were introduced into each of two identical chambers, providing a group of experimental and a group of control subjects. These groups were maintained in the ant room, where external disturbances were minimized. Twice each day they were taken to the x-ray room, left for several minutes, then returned to the ant room. This procedure was intended to habituate the test subjects to the complex cues of handling the containers, noises and scents of the x-ray room, etc., that would unavoidably be associated with delivery of the US.

During this phase a daily preference score for the rough-bottomed compartment (which was to be exposed to x-rays) was obtained according to the method outlined above.

Exposure phase - On the evening of day three, experimental and control groups were taken into the x-ray room, exposed for a pre-determined rate and duration (or sham-irradiated for a comparable period), then returned to the ant room. Sham exposure involved procedures identical to true exposure except that the power was not applied to the x-ray tube.

Post-exposure test of conditioning - Careful attention was paid to the distribution of ants within the chamber. The daily preference score (above) and other pertinent aspects of general behavior were recorded for as long after exposure as deemed worthwhile, usually several days.

Variations in the above protocol included (1) several successive exposures immediately following an initial exposure, the time of delivery of each being determined by the behavior of the experimental subjects, (2) successive daily exposures at the rate of one per day, and (3) exposing ants individually (one ant per chamber) rather than in groups.

Surgical Procedures

In certain experiments on avoidance behavior it was necessary

to subject antennectomized and ophthalmectomized ants to x-rays

within the free-choice chambers. Operations were performed in the

manner described in the section on Studies of Prompt Detection.

Lethality Studies

A study was made to determine the amounts of x-irradiation

necessary to incapacitate or kill ants in relatively short periods of

time. Conditions of exposure, including the use of single-compart-

ment exposure chambers (Figure 4), were identical to those des-

cribed above in the section on Studies of Prompt Detection. All ex-

posures were made at 25 R/sec, and each of five groups received a

different total exposure, ranging from 25 kR to 150 kR. The behav-

ior of ants during exposure was viewed on closed-circuit TV, but

was not recorded on videotape.

For a period of 27 weeks after irradiation, I made observations

on behavior and recorded the distribution of deaths in time at each

exposure level. Similar observations were made for a group of con-

trols that was sham-irradiated.

The lethality data were subjected to quantal response analysis

by means of a computer program, adapted to the Oregon State Univ-

ersity CDC 3300 by Hodge (1970). The computer program derived

the best fit line through a maximum-likelihood method described by

Nachtwey, Ainsworth, and Leong (1967). The program operates by

transforming the number of responses (deaths, in this case) at each

dose to a weighted normal equivalent deviate (NED) value (NED =

probit minus 5); then the linear regression is analyzed for the maxi-

mum liklihood of being the best fit line. New weights are assigned

and the computation is iterated with new weighted NED values. Test-

ing, weighting, and iteration continue until the values for the Y inter-

cept and the slope of the regression line do not differ from the pre-

vious values by more than 0.001. The confidence limits are based on

a statistical parameter for probability 0.05.

RESULTS

Prompt Behavioral Responses to X-Rays

Intact (Normal) Subjects

Two characteristic behavior reactions occurred through a wide range of exposure rates and beam qualities. (1) Antennal waving was the most frequent response. The movement of both antennae was such that the tips cut wide arcs through a vertical plane. This behavior appeared to represent a taxis or orientation response. Antennal waving was always the first response given to x-rays, and often was the only response at low exposure rates (less than 0.5 R/sec). (2) Running behavior was also a characteristic prompt response. At moderate to high exposure rates (1 R/sec and above), antennal waving was consistently accompanied by a burst of running activity along the floor, sides, and top of the exposure chamber.

The latency, intensity (strength), and duration of prompt responses were characterized as a function of exposure rate. Prompt responses were faster and stronger in subjects tested in the spring and summer than in subjects tested in the fall of the year. These seasonal differences will be considered later. The results which follow are all based on work done between April and August.

Latency of Prompt Responses

The latency of both the antennal-waving response and the running response was an inverse function of the exposure rate. Figure 9 illustrates this principle for the antennal-waving response. Note that the latency varied through three orders of magnitude. As mentioned earlier, the lower exposure rates could be achieved only by using different beam qualities, which are indicated by different symbols in, and explained in the legend of, Figure 9. There was no significant difference in the average latency to response at points of common beam qualities (10 R/sec, 1 R/sec, and 0.1 R/sec; Figure 9); thus the curve in Figure 9 was fitted to an average value at each exposure rate where more than one beam quality was employed.

Very rapid (less than one second) antennal responses were given by all subjects at exposure rates of 10 R/sec or greater. The duration of exposure necessary to elicit this response from at least half of the subjects being exposed was about seven seconds at 0.1 R/sec. Below about 0.05 R/sec (3 R/min) no ant gave any behavioral evidence of detection, regardless of the duration of exposure (see tabular data, Figure 9).

At exposure rates of 10 R/sec and above, the running response began simultaneously with the antennal response, and both responses were exhibited by 100 percent of the subjects. Between 1 and 5 R/sec,
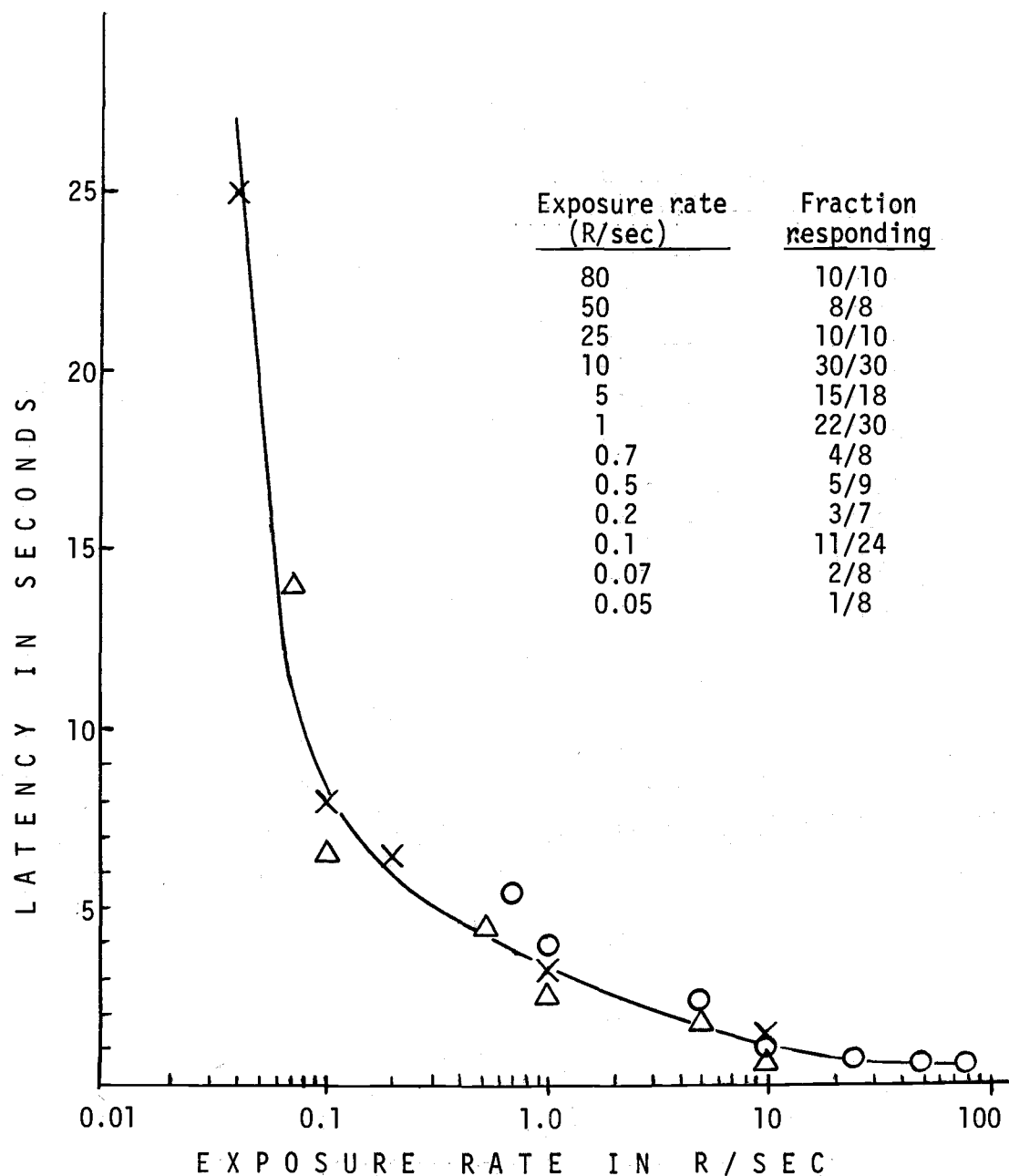
Figure 9. Latency of the antennal-waving response (graph), and the
fraction responding (tabular data), as a function of
exposure rate. Each point (symbol) except the one at
0.05 R/sec is an average of 2-12 subjects. Different
symbols represent different beam qualities:

O = 300 kV, 20 mA, 1.8 mm Al. HVL
X = 300 kV, 20 mA, 4.0 mm Cu. HVL
△ = 100 kV, 20 mA, 0.25 mm Al. HVL

the running response usually occurred one to several seconds after a subject began waving its antenna. Rates of less than 1 R/sec often evoked only the antennal response; when running did occur it was typically several seconds later in appearance than the antennal-waving response. Running was never seen to occur within a 30 second exposure period at rates of 0.2 R/sec or less.

Intensity of Prompt Responses

The intensity of the running response was a direct function of exposure rate. Speed of running, taken as a measure of the strength (intensity) of the running response, is plotted as a function of exposure rate in Figure 10. Speed of running appeared to be independent of exposure rate between 0.5 and 5 R/sec, then increased dramatically up to 80 R/sec. Rates below 1 R/sec did not consistently elicit running behavior from all subjects tested (but detection was evidenced by antennal waving).

I did not attempt to quantify the change in intensity of antennal waving at different exposure rates, because once subjects began running rapidly it was not possible to follow their antennal movements.

Duration of Prompt Response

Some ants exhibited adaptation during exposure at rates below 0.5 R/sec, i.e. the antennal response waned and subjects ceased to
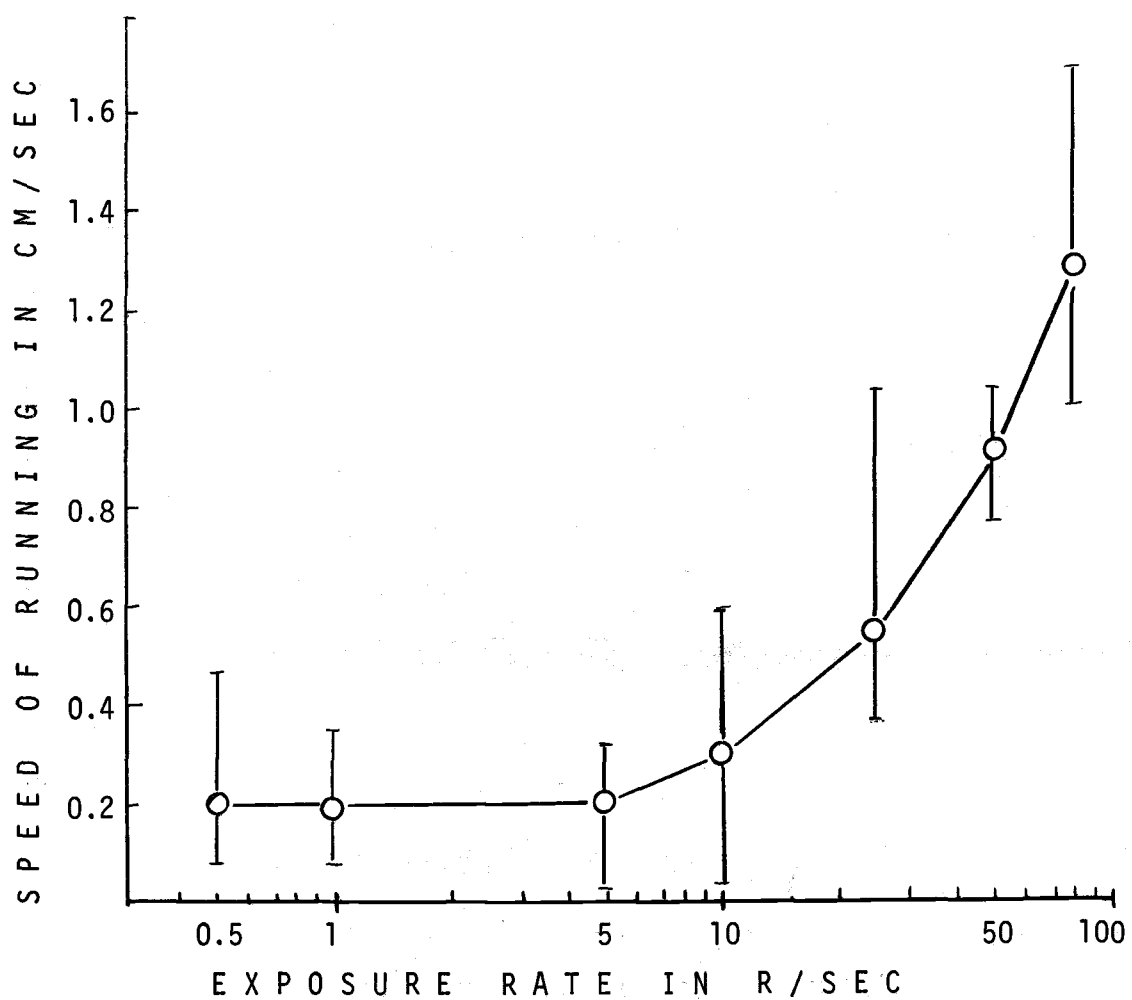
Figure 10. Average intensity of the running response as a
function of exposure rate. Each point is an
average of 5-8 subjects. Vertical bars show range
of response.

give behavioral evidence of detection while the beam was still on.

Rates of 1 R/sec or higher, however, usually caused antennal waving

and running to persist throughout the exposure period. During long

(minute) exposures, ants would periodically stop running and clean

their antenna, but these brief pauses were transient. Rapid running

was quickly resumed, and there were no clear indications of adapta-

tion, or habituation at exposure rates of 1 R/sec or higher. This

continuous, high-level activity is illustrated for protracted exposure

at three different exposure rates in Figure 11.

On termination of exposure, most subjects exhibited an abrupt

cessation of running, accompanied by vigorous antennal-cleaning be-

havior. Thus, both the onset and the cessation of irradiation elicited

detectable changes in the behavior of ants. At rates of 10 R/sec or

higher, the "off" response was transient, lasting only a few seconds.

A high level of activity then reappeared, even though the beam had

gone off. Intensity of post-exposure running often approached that

exhibited during exposure (see Figure 11), and persisted for several

minutes. At high exposure rates, radiation obviously induced an in-

tense state of excitation, that lasted well beyond the period of ex-

posure. This phenomenon is unrelated to prompt responses, and is

dealt with in a later section.

A test was made to determine the effects on prompt behavioral

responses with a series of repetitive exposures. Alterations in the
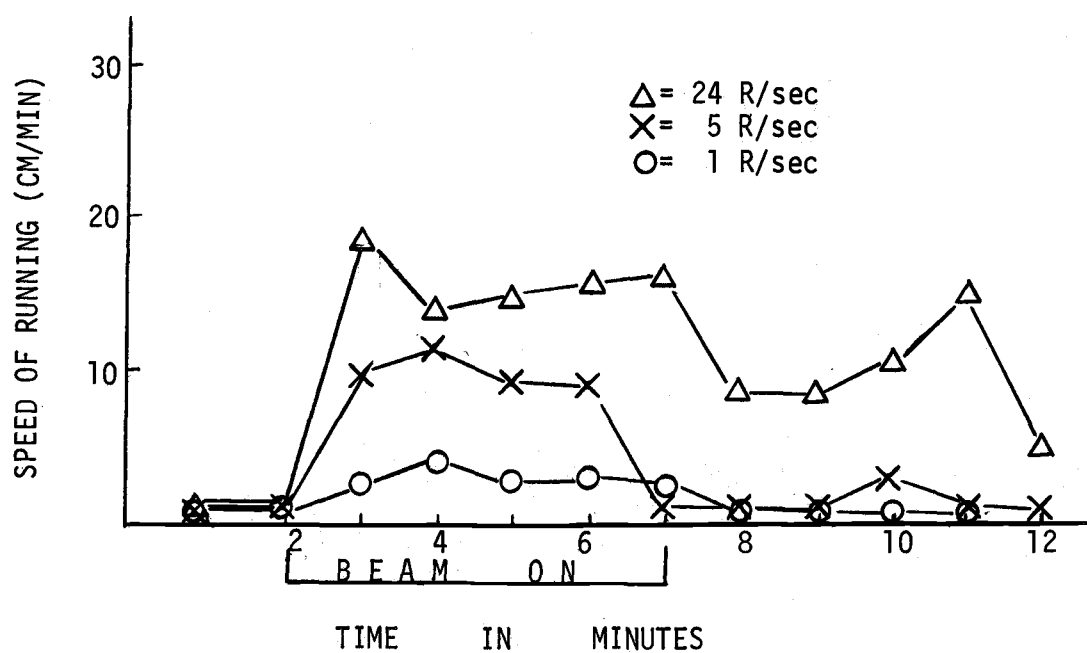
Figure 11. Strength and persistence of the running response as a function of exposure rate where the duration of exposure was extended to five minutes. Each point is an average of 3-5 individuals.

response pattern might result from sensitization, fatigue, or direct tissue damage. A group of 10 ants was subjected to six exposures during a two-day period. Each exposure, at 80 R/sec, lasted for one minute. There was no measurable change in the latency or the strength of prompt responses with successive exposures. However, the duration of post-exposure activity increased somewhat with each exposure, suggesting a dose-dependent aversive quality, which will be considered later.

Ophthalmectomized Subjects

The prompt nature of the foregoing responses indicated that the nervous system was being stimulated, either directly or indirectly, possibly through specific sensory pathways. A previous study (Smith, Kimeldorf, and Hunt, 1963) demonstrated that immediate motor responses of moths to x-rays occurred only when the eyes of the moth were in a dark-adapted state. My results had been obtained with ants exposed in a well-lighted room, casting doubt on the hypothesis that visual detection might be responsible. Nevertheless, the higher exposure rates used in the present study could conceivably have stimulated eyes in a light-adapted state. To test this possibility, I bilaterally ophthalmectomized two groups of ants (n = 5/group) and exposed them to 10 R/sec for 30 sec. Responses were just as strong as those given by controls, and while the average latency of the running

response was somewhat longer in eyeless subjects (1.2 seconds) than in normal ants (0.9 seconds), this difference was not statistically significant. Thus, the visual system was not primarily responsible for the prompt behavioral reactions given by ants to brief exposures of x-rays.

Antennectomized Subjects

Insect antenna are abundantly laden with a variety of sensory nerve endings and, as distinct appendages, are readily available for experimental manipulation. Several experiments were performed in an attempt to elucidate the role of the antenna in prompt responses given by ants on exposure to x-rays.

When stimulated at 10 R/sec or higher, antennal waving and running behavior began simultaneously, usually within one second of the onset of exposure. To determine whether intact antenna were essential for the running response to be elicited, I removed either one or both antenna from several subjects, then exposed them to 10 R/sec for 30 seconds. The running responses of six unilaterally antennectomized subjects were identical to responses of normal subjects. In contrast, none of six bilaterally antennectomized ants gave any detectable response during exposure. Antenna thus appeared essential for the detection of a stimulus which led to the running response given by normal subjects.

It was possible, however, that antennoctomy debillitated ants such that they were physically incapable of responding to any stimulus. Ants without antenna were generally less active than controls, but did eat and drink, and lived for several weeks. In addition, Karl von Frish (1950) showed that complete antennectomy did not interfere with locomotion and visual discrimination in bees, which are closely related to ants. However, to insure that antennectomy had not physically disabled ants, I compared the responses of five normal and five antennectomized subjects to a mild electric shock (see Methods). The threshold voltage required to elicit a response was the same for experimental and control groups, ranging from 2.5 to 5 volts. Also, the responses of both groups were remarkably similar: aggressive displays, jumping, and, at higher voltages, wild running and escape behavior. Ants deprived of normal sensory input from their antenna were still capable of responding to a stimulus that did not involve the antenna.

Next, I amputated only the segmented, flagellar portion of both antenna from each of six ants, then exposed them to 10 R/sec for 30 seconds. No ant gave any indication of detection. Thus the flagella were the sensitive portions of the antenna for the induction of radiation-induced prompt behavioral responses.

In the type of antenna possessed by ants, aggregations of specialized sensory nerve endings are found only in the flagella. By

contrast, antennal muscles and motor components of the large antennary nerves are restricted to the scapes (Schneider, 1964). The sensory component of the nerve tract extends from the deutocerebrum through the scapes into the flagella. Since ants with scapes intact did not respond to x-rays (above), one can presume that ionizing radiation must act primarily or exclusively on the receptor neurons distributed along the segments of the antennal flagella.

A study was made to determine which of the 11 flagellar segments were involved in detection. Eleven experimental (n= 10/group) and one control groups were prepared. Members of each experimental group had "n" segments removed from both antennal flagella. The "n" was equal to one segment for the first group, and increased by one for each succeeding group, such that ants in group 11 had all eleven segments (the entire flagellum) missing from both antenna. Each ant was individually maintained and exposed to 10 R/sec for 30 seconds. Latency and strength of behavioral responses were scored, as well as the percent responding to exposure.

The latency of the running response is plotted in Figure 12 as a function of the number of segments removed. Percent subjects responding is also tabulated in this figure. Note that nearly all ants in each group missing fewer than six segments responded as quickly as did the controls. Most ants missing 6, 7, or 8 segments did respond, but the average latency of the response increased markedly. Almost

Number of segments off	Percent responding
0	100
1	100
2	100
4	90
5	100
6	90
7	90
8	70
9	10
10	20
11	10

LATENCY IN SECONDS

FLAGELLAR SEGMENTS REMOVED
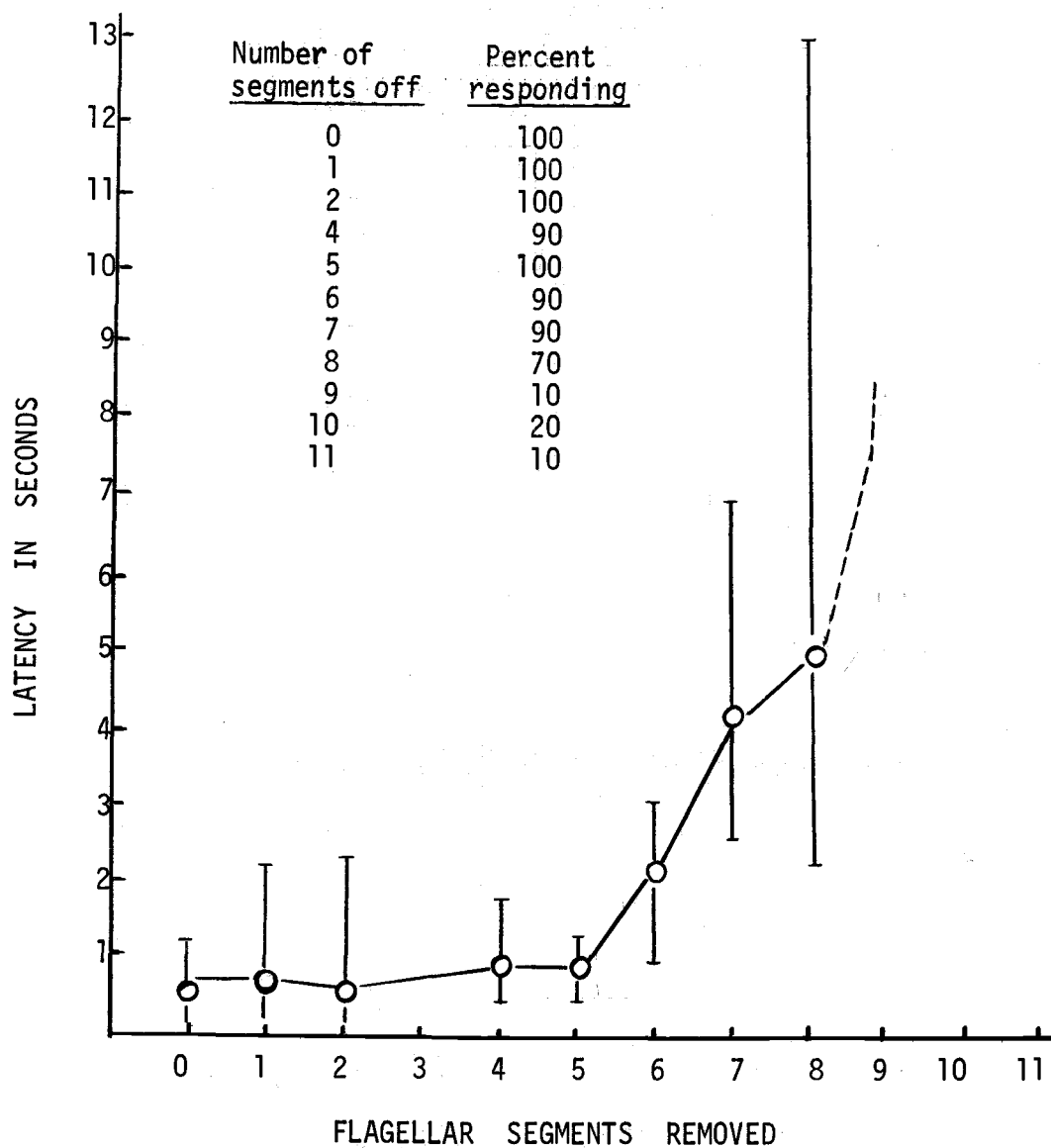
Figure 12. Latency of the running response as a function of the number of segments amputated from both antennal flagella. Vertical bars show range (n= 10/group). Exposure rate, 10 R/sec. Percent subjects responding is given in tabular form.

none of the subjects missing nine or more segments gave evidence

of detection during the exposure period (in other words, latency to

respond became infinite). These results are also illustrated dia-

grammatically in Figure 13.

Although the strength of prompt responses (frequency of anten-

nal waving and speed of running) was not quantified, this parameter

followed the same pattern as latency. While ants with more than

six segments intact responded as strongly as did the controls, when

the sixth, seventh, and especially the eighth segments were missing

subjects usually signalled detection by mild waving of the antennal

"stubs", and moving slowly around.

These results showed that carpenter ants deprived of sensory

input from certain of the distal segments of the antennal flagella

either did not detect a stimulus, or the stimulus was not sufficiently

strong to cause immediate, transient behavioral responses typically

given by normal subjects upon exposure to x-rays.

The next question concerned the specific kinds of receptors involved.

Antenna contain sensory receptors specialized for olfaction, mech-

anoreception, hygroreception, and thermoreception (Schneider, 1964).

While receptors for most senses are also found elsewhere on the

body, antennal flagella are the principle site of olfactory receptors

(as distinguished from gustatory or contact chemoreceptors). In

1921, Karl von Frisch (see von Frisch, 1950) demonstrated
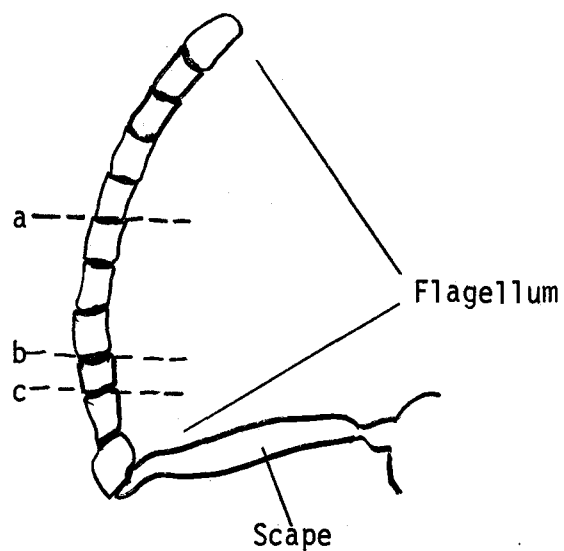
Figure 13. A diagram relating the effects of segment amputation
to the occurrence of prompt behavioral responses to
x-rays (10 R/sec). Amputation of segments distal to
line (a) had no effect; progressive removal of seg-
ments between (a) and (b) resulted in increasing
latencies and decreasing strength of responses; cut-
ting along line (c) abolished all prompt responses.

unequivocally that the honey bee's reactions to odors are lost when

the distal eight segments of the flagella are amputated, and similar

results have been obtained from behavioral and electrophysiological

studies on other insects (Schneider, 1964).

To test the hypothesis that olfactory receptors were responsible

for detection of x-rays by ants, 12 groups of ants were prepared for

tests with various odors (n = 3-5). As in a previous experiment,

members of each group had "n" segments removed from both flagella,

where n = 0 for the controls and increased by one for each succeeding

group. Ants were individually presented with the odor of a foreign

ant, then the odor of a nestmate. Results were striking; most ants

missing up to nine segments reacted aggressively toward the foreign

odor, attacking or attempting to escape from the source. These sub-

jects all accepted or ignored the odor of a member from the same

nest. In sharp contrast, ants with nine or more flagellar segments

missing were not capable of discriminating between the odors of

"friend" and "foe". Indifference to both odors was the most common

response, but if an experimental subject rejected (attacked) one odor,

it also rejected the other. Thus, ants with up to nine segments re-

moved from their antennal flagella responded to x-rays (Figure 13)

and were also capable of odor discrimination. Those with nine or

more segments amputated did not respond to 10 R/sec and could not

make the odor discrimination. These results suggest that olfactory

receptors participate in the detection of x-rays which result in prompt behavioral reactions of carpenter ants.

Ionizing radiation might have (1) constituted an effective stimulus for flagellar receptors, or (2) acted indirectly via the production of ozone, or other radiolytic byproducts in air, which in turn stimulated flagellar receptors. I reasoned that an impervious coating of shellac on the antenna would inhibit the potential action of ozone, yet be easily penetrated by x-rays. Accordingly, the antenna of each of 10 ants were shellaced, after which ants were tested for and found incapable of discriminating between odors. Ants were then individually exposed to 10 R/sec for 30 seconds. Seventy percent of the experimental subjects responded by running behavior within five seconds, but the average latency of response, 1.7 seconds, was twice that of normal subjects. Speed of running was highly variable, and generally weaker than that of controls.

The reduced responsiveness of these experimental subjects might mean that ionization products in air contributed to the fast, strong responses of normal subjects. However, that ants with shellaced antenna did respond suggests that the response was due, at least in part, to x-rays acting in or on the immediate surface of the antennal receptors.

While the prompt responses of light-adapted ants appeared to be mediated entirely by antennal receptors, it seemed possible that

dark-adapted ants might respond to x-rays through visual stimulation. To test this hypothesis, 10 antennectomized ants were exposed to 10 R/sec for 20 seconds after the lights in the x-ray room had been dimmed such that I could barely see the outlines of subjects on the TV monitor. No behavioral responses were observed to occur in any subject.

Bioelectric Responses of Sensory Receptors

The Isolated Eye

The results of behavioral studies illustrated that a visual route of detection need not be postulated to explain prompt behavioral re- actions to x-rays. However, the isolated ant eye was found to promptly detect stimuli of ionizing, as well as non-ionizing radiation. Electroretinograms were obtained with light, x-ray, and beta-ray stimuli. Results of the light-induced ERG will be presented first, to provide a base against which responses of the eye to ionizing radia- tions can be compared.

Light-Induced ERG

A rapid increase, then decrease, in light intensity evoked correspondingly rapid changes in the electrical potential within the eye, resulting in an ERG (Figure 14). A slow increase in light
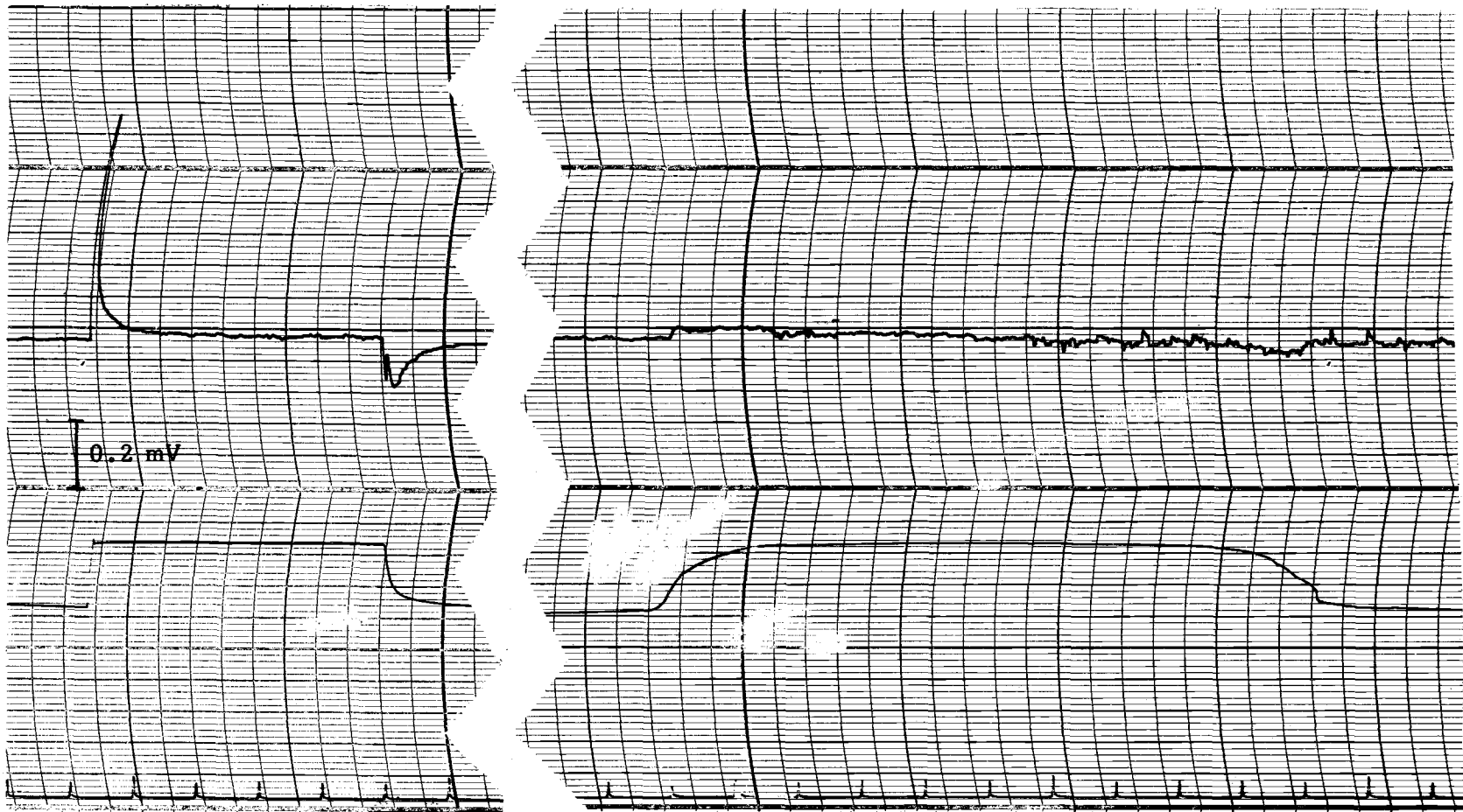
Figure 14. An ERG response, showing "on" and "off" peaks (left), and suppression of response to slowly-changing intensities (right). Upper tracing, ERG response, with initial upward deflection being corneal-negative "on" potential. Middle tracing, stimulus marker showing onset and duration of stimulus. Lower tracing is time record, in seconds. Light stimulus: 500 nm, 5.78 X 10^3 ergs/cm^2-sec.

intensity did not elicit an ERG, even though the same absolute change in intensity occurred in both cases (Figure 14).

Several typical responses to stimuli of different durations are shown in Figure 15. Regardless of stimulus duration, the "on" response (upward deflection) fell rapidly to the baseline, and then upon cessation of stimulation, a deflection of opposite polarity ("off" response) appeared. When the stimulus duration was sufficiently short, the "on" and "off" responses merged imperceptably (third peak from left). Note also that the amplitude of all response peaks is nearly the same. The right portion of Figure 15 is a recording, at slower chart speed, of ERGs to flashes delivered at a much faster rate, illustrating the decrease in amplitude of successive responses when the interstimulus interval is less than the complete recovery time of the eye. Note that the decrease in response amplitude ceased after about 10 seconds, and the eye continued to respond at reduced strength for an indefinite period.

The strength (amplitude) of the light-induced ERG was a direct function of stimulus intensity. This relationship will be illustrated below, in comparison to x-ray ERGs, in Figure 18.

X-Ray-Induced ERG

All eyes exposed to x-ray stimuli gave measurable ERGs that were remarkably similar to those elicited by light.

Figure 15. Responses to a series of 500 nm light stimuli of varying durations (left), and decrease in response upon reduction of interstimulus interval (right). Tracings as in Figure 14. Note that in the right record the stimulus marker was inoperative; flash rate and duration, 2.5/sec and 1/25 sec, thus exposure/flash = 230 ergs/cm^2.

Rapid increases and decreases in intensity resulted in sharp "on" and "off" responses, whereas a slowly-changing intensity was ineffective in causing a response (Figure 16). A series of responses to stimuli of various durations are seen in the left part of Figure 17. This record shows that, as with visible light stimuli, an x-ray stimulus modified the responsiveness of the eye to subsequent stimuli. If the interval between stimuli was too short, the eye responded at successively reduced strength; full recovery occurred within one second after temination of a train of rapid stimuli, as manifest by a subsequent response of maximum amplitude (middle of left record, Figure 17). The right record in Figure 17 shows that this decrease in response leveled off within several seconds, after which the eye continued to respond indefinitely, at reduced strength.

The strength of the ERG response was a direct function of the strength of the stimulus, for both x-rays and light. Amplitude-intensity curves for both stimuli exhibit similar slopes through the entire range of intensities used in this study, as shown in Figure 18. This and other similarities of x-ray and light-induced ERGs strongly suggests that similar mechanisms underly the ERG response in the case of both stimuli.

The threshold exposure rate of x-rays to elicit a bioelectric response from the eye was below 300 mR/sec, which was the lowest exposure rate used in this study (Table 2; Figure 18). At this rate
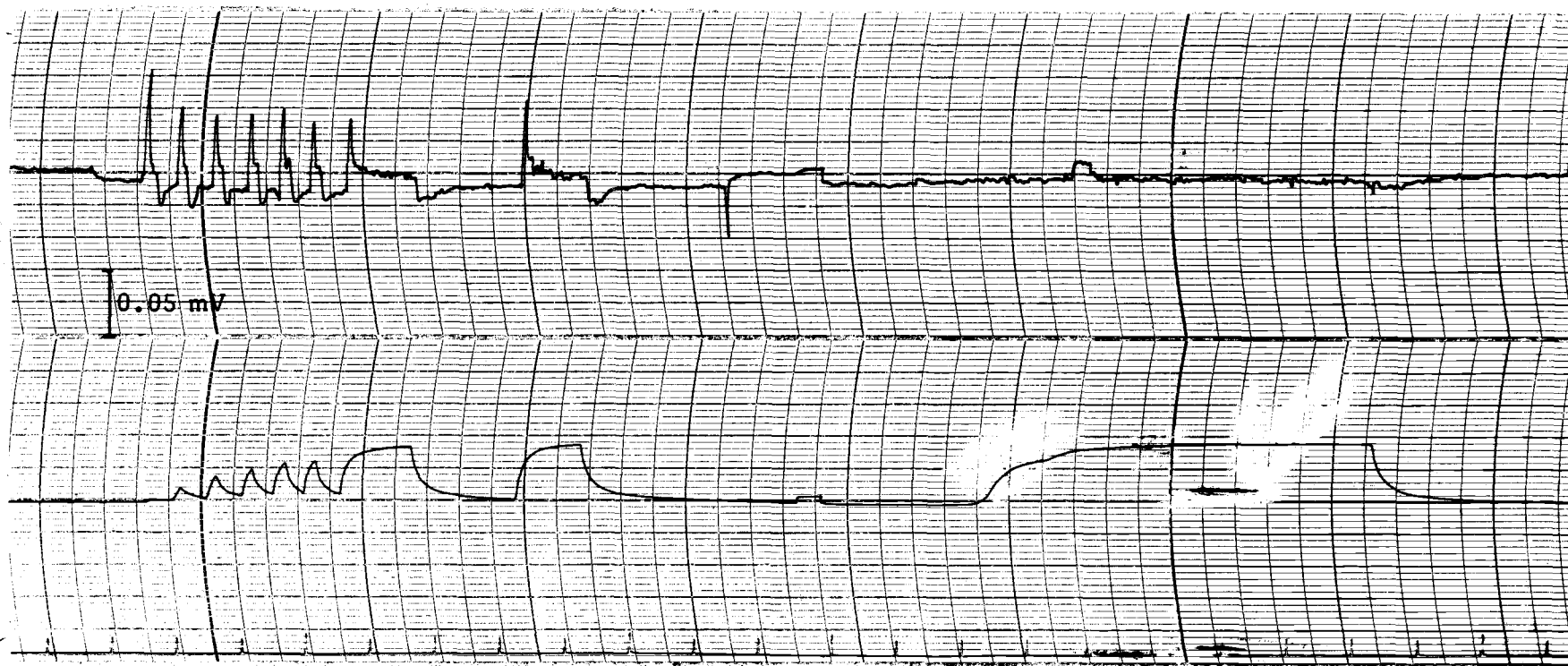
Figure 16. Typical ERG "on" and "off" responses to rapidly-changing intensities of x-rays, followed by demonstration that slowly-changing intensities failed to elicit the ERG. Tracings as in Figure 14. Deflections in center are artifacts of machine operation. Exposure rate, 2.25 R/sec.
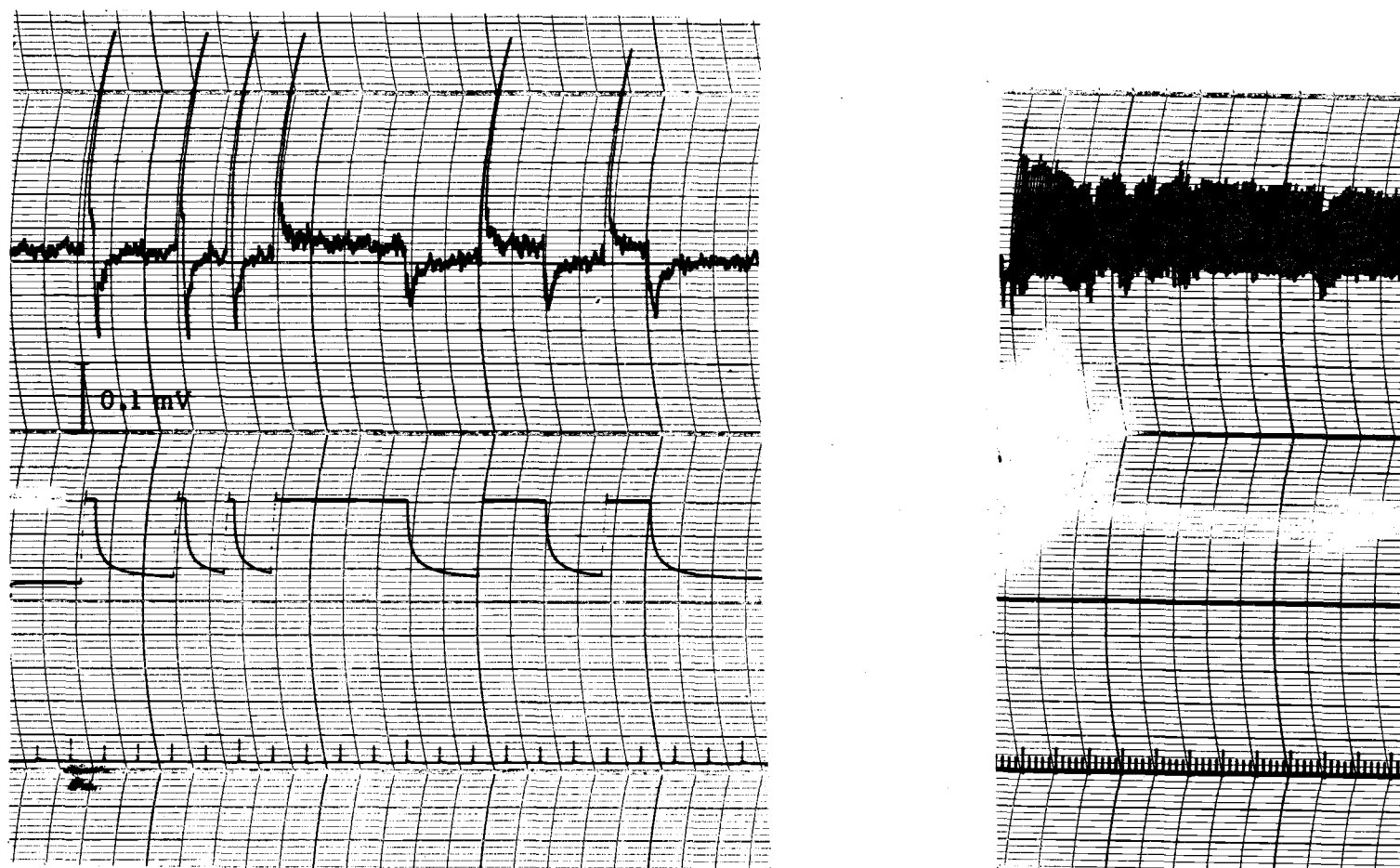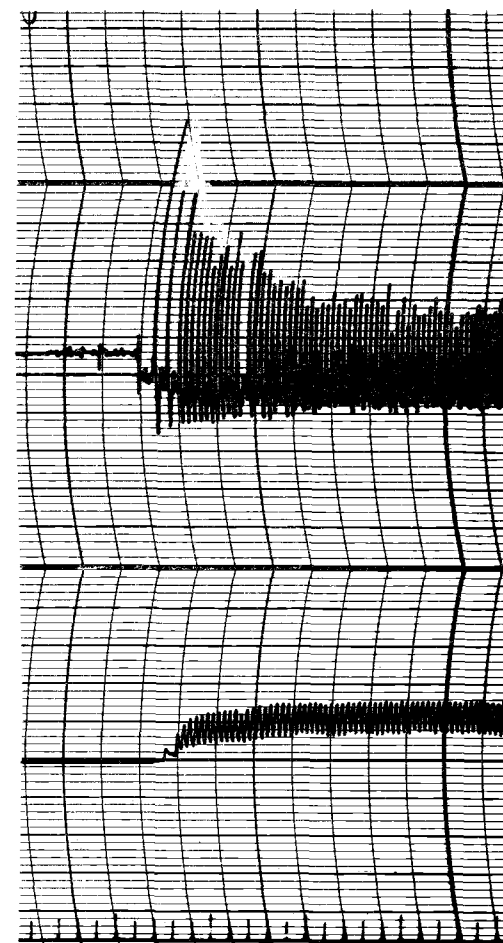
Figure 17. Responses to a train of x-ray flashes of varying durations (left), and decrease in response upon reduction of interstimulus interval. Tracings as in Figure 14. In the right record, flash rate and duration were 3.5/sec and about 1/10 sec. Exposure per flash, 0.22 R.
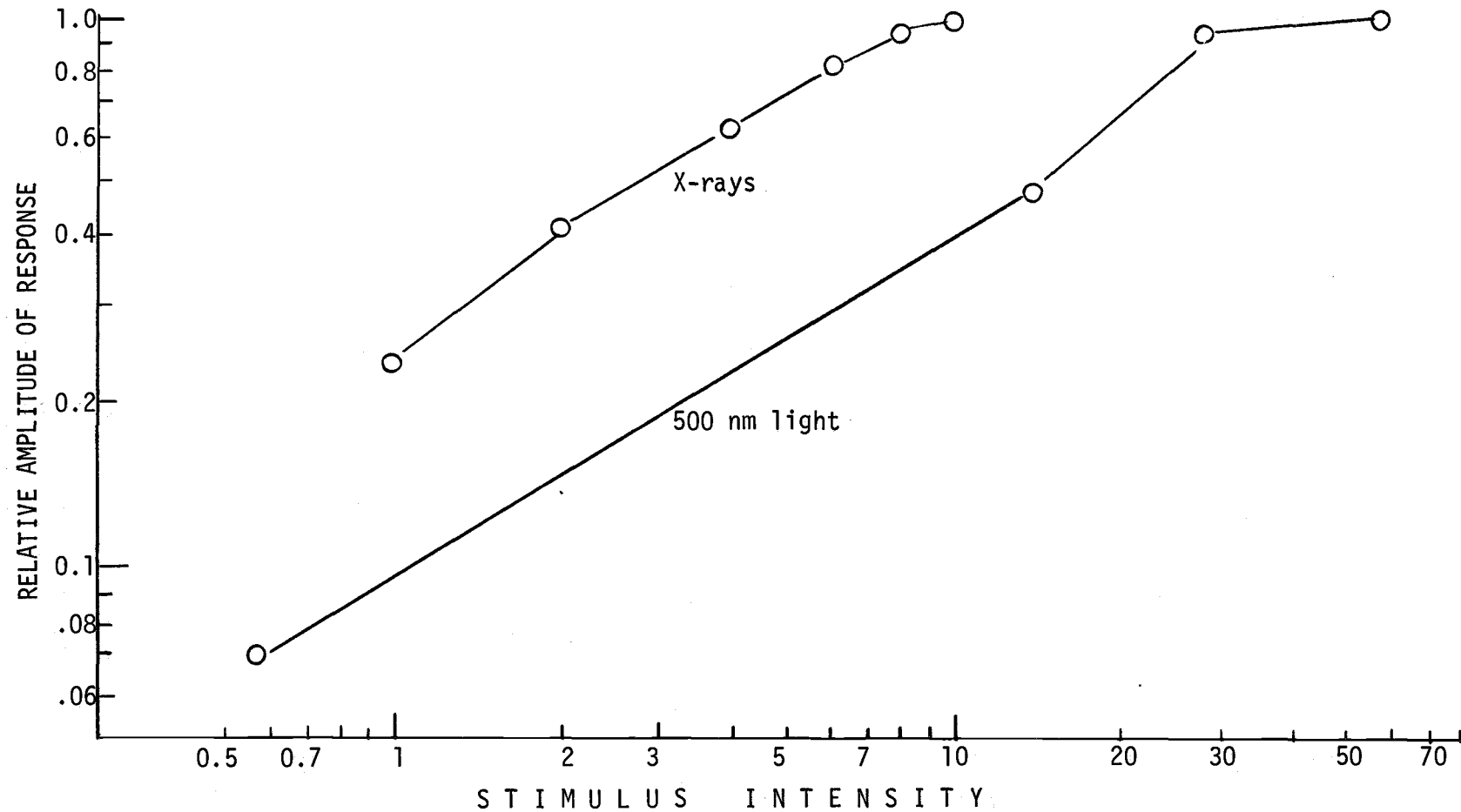
Figure 18. Strength (amplitude) of ERG response as a function of stimulus intensity, for x-rays (in milliamps; see Table 2), and for visible light (ergs/cm^2-sec X 10^2). Maximum amplitude for each stimulus was normalized to 1. Each point is an average of two trials on two different eyes. Duration of exposure was constant for all trails.

almost all eyes gave small but distinct peak responses, even when the flash duration was only 100 milliseconds, resulting in an exposure per flash of 30 mR.

Beta-Induced ERG

Only five of nine eyes tested responded to beta radiation with measureably ERGs. Typical responses to a series of beta stimuli (7.7 mrads/flash) are illustrated in Figure 19. Note that, although the upward deflection of each "on" response rose promptly with the onset of each stimulus, it decayed back to the baseline relatively slowly, and there was not a conspicuous "off" response.

There was a decrease in the amplitude of responses to each succeeding stimulus, but this decrease differed from that given on stimulation with light or x-rays in three ways. (1) The decrease continued until the response peaks merged imperceptably with the noise level of the amplifier and recorder (Figure 19). Figure 20 is a graph of this response decrease to beta stimuli, to x-ray stimuli, and to light stimuli. The shapes of the curves suggest that the mechanisms of action on the eye may be different for beta particles than for the other two stimuli. (2) Once the eye ceased to give measurable responses to beta stimuli, no further responses could be elicited, even after up to one hour of rest. An obvious assumption was that beta radiation was damaging the eye, but repeated tests showed that
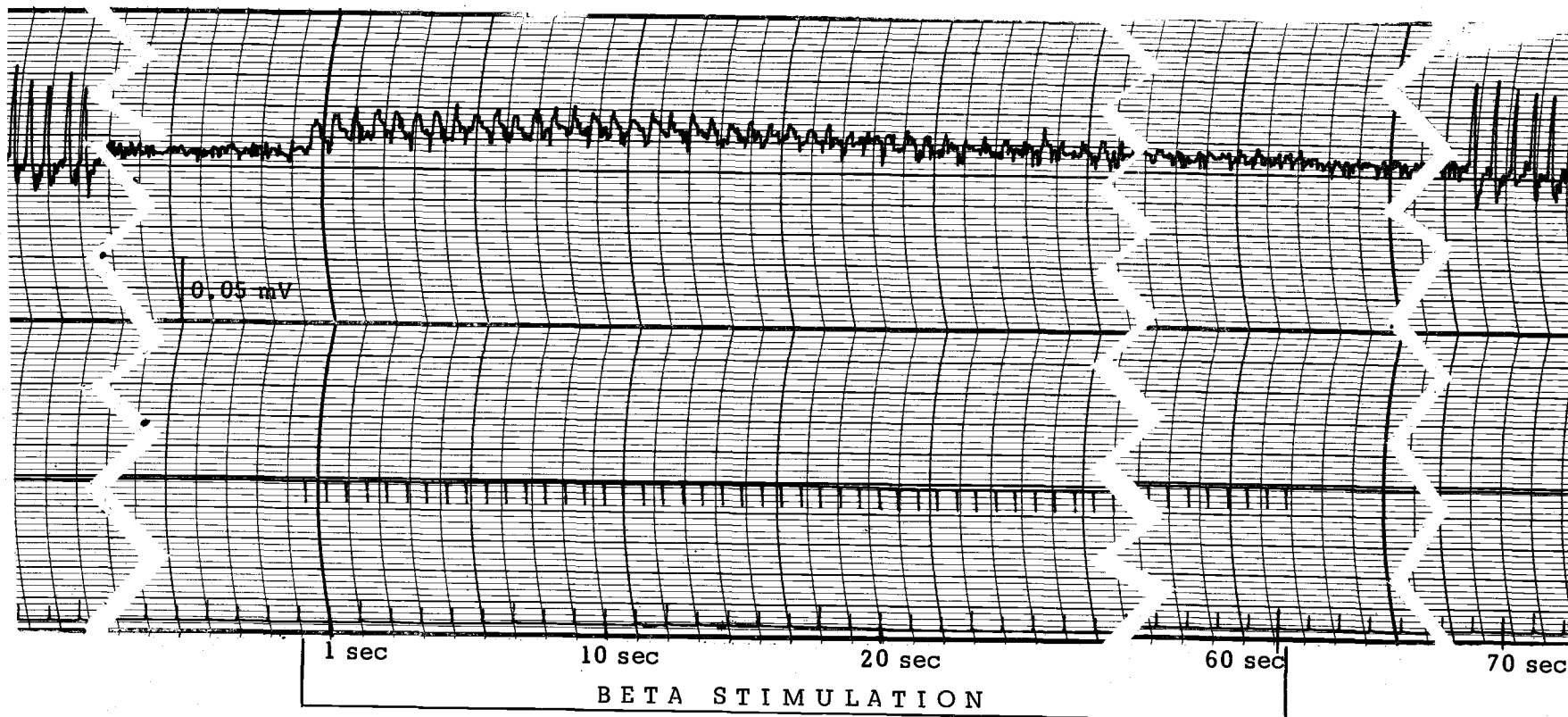
Figure 19. ERGs to a series of beta stimuli, preceeded and followed by responses to rapid flashes of light. Traces as in previous records. Stimulus conditions: 7.7 mrads per 1/30 sec flash, 1.6 flashes per second (see Table 1 for clarification).

Stimulus	Flashes Second	Msec Flash	Exposure Flash
Light	2.5	40	230 ergs/cm^2-sec
X-rays	2.8	100	220 mR
Beta particles	2.0	25	5.8 mrads
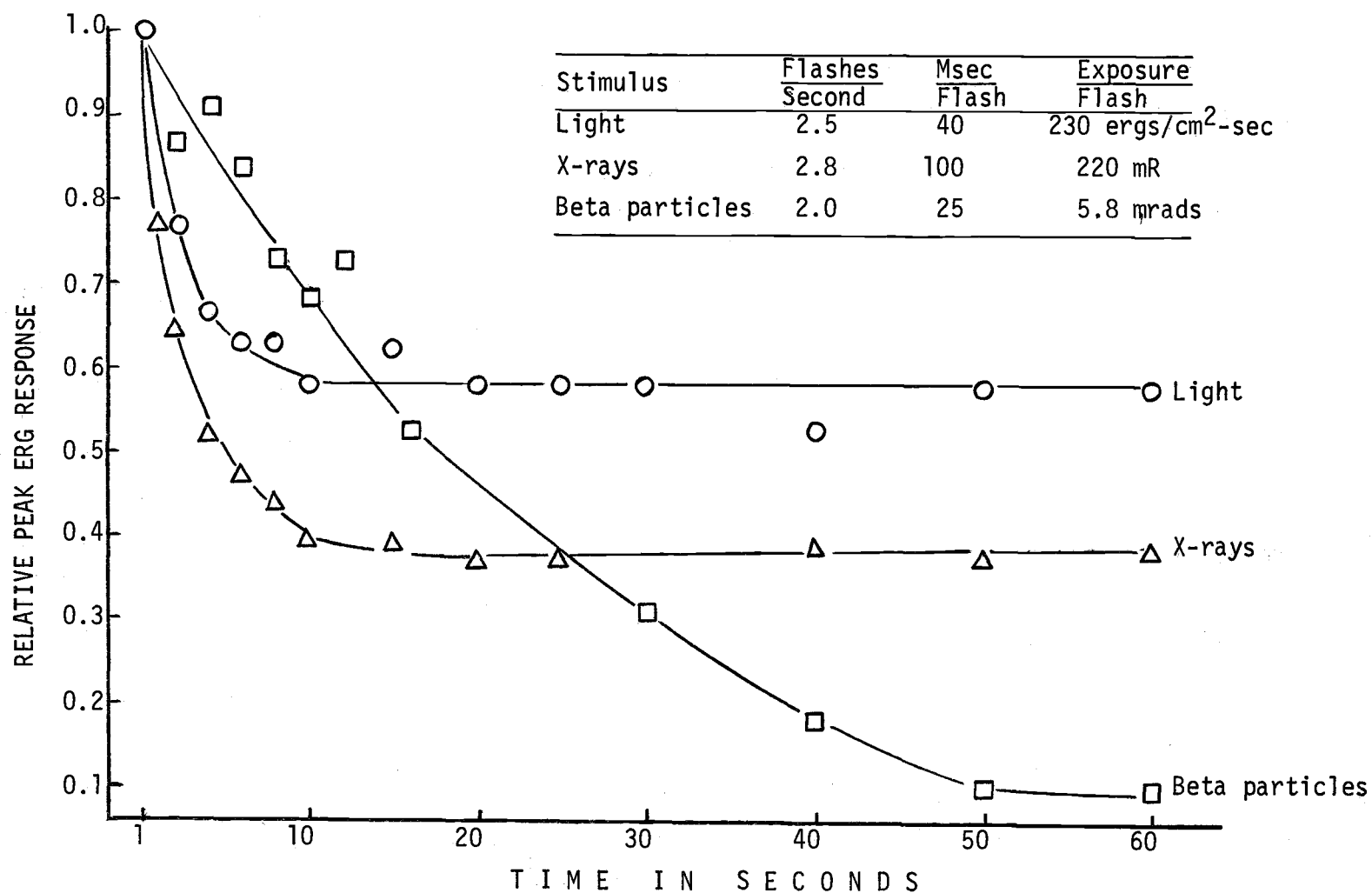
Figure 20. Response decrease of ERG to rapid series of flashes for all three types of radiation. Each point is an average of comparable responses from at least two eyes. Stimulus conditions are given in box.

such an eye always responded immediately and strongly to light stim-

uli (compare the initial and final light-induced ERGs in Figure 19).

Thus beta stimulation, while rendering the eye measurably insensitive

to subsequent beta stimuli, did not inactivate the photoreceptive

mechanism that was responsive to light. (3) The duration of the inter-

stimulus interval ("rest" period) had no effect on the decrease in re-

sponses to successive beta stimuli. For example, when the stimulus

rate was doubled by increasing the speed of the rotating shutter,

which also halved the stimulus duration and the interstimulus inter-

val, the same pattern of response decrease was observed. Further,

if a train of beta stimuli was terminated prior to complete loss of

responsiveness, then resumed several minutes later, the decline in

amplitude progressed as if the two trains had been delivered uninter-

rupted. Thus, the loss of response to beta stimuli seemed irrevers-

ible, and appeared to depend on total absorbed dose, although this

relationship was not investigated in detail.

Amplitude-intensity studies were not done systematically be-

cause the permanent loss of responsiveness made it impossible to

use an eye as its own control. However, the initial ERGs of eyes

which did respond to flashes of beta radiation were stronger at higher

dose rates than at lower dose rates. The threshold dose rate re-

quired to elicit an ERG was approximately 62 mrads/sec. At this

dose rate, a train of stimuli from the 50 mCi source elicited small

but distinguishable response peaks from one of the eyes studied when the flash duration was 1/20 sec (3.1 mrads/flash), but not when it was decreased to 1/40 sec (see Table 1).

In spite of the unexplainable differences between x-ray and beta-induced electroretinographic responses, these studies clearly established that, under conditions of dark-adaptation, the eyes of ants detected the presence of high-energy photon and electron stimuli. Such detection apparently did not initiate the prompt behavioral reactions of ants to x-rays, but observations made while studying behavioral responses to prolonged exposures (to be discussed) indicated that visual detection is involved in the "off" response elicited upon termination of exposure.

The Isolated Antenna

Electroantennograms were obtained from several isolated antenna upon exposure to the odor of a foreign ant, the odor of formic acid, and to beta radiation. Representative records of EAGs will be presented, and comparisons made, in the results which follow.

Odor-Induced EAG

Six of eight antennae responded to the odor of formic acid, which is an excitatory pheromone elaborated by ants under natural conditions. The large, upward deflections in the top record in Figure 21 each

Figure 21. Representative EAGs from one isolated antenna exposed
to three different stimuli. For each record, upper trace
is the EAG, lower trace is the time in seconds. Solid
blocks beneath time marks represent the onset and durat-
ion of stimuli. Sham tests follow,or, in the bottom record
preceed, the true stimulus. Deflections between expos-
ures and sham tests are artifacts related to operation of
the amplifier and recorder.

ODOR OF FORMIC ACID

ODOR OF FOREIGN ANT

BETA RADIATION
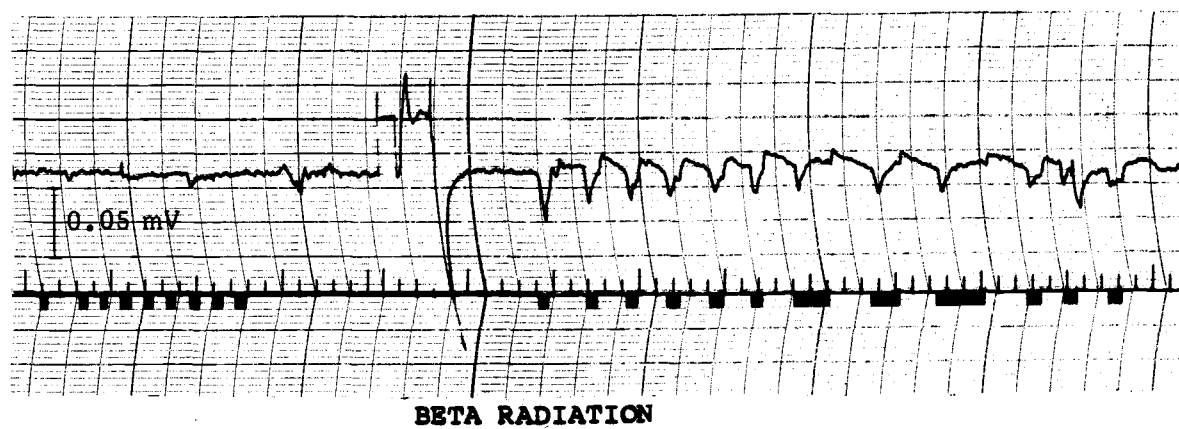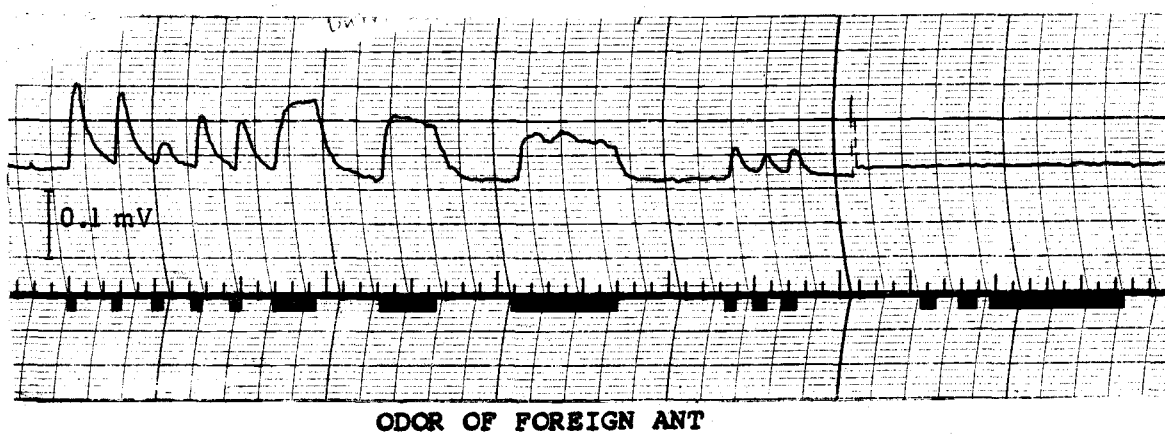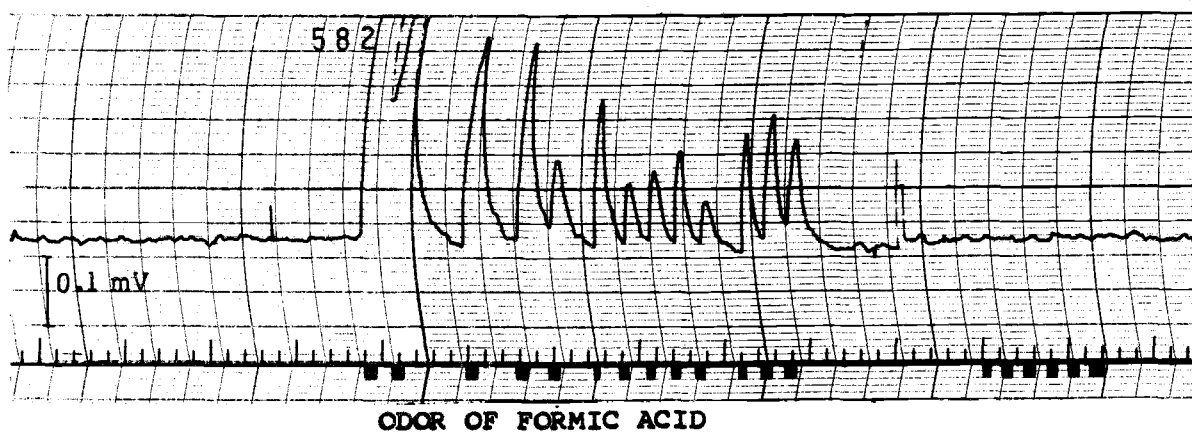
correspond to a 400 - 800 millisecond exposure to this odor, followed by an illustration that sham tests to the "odor" of distilled water did not result in responses. An upward deflection means that the tip of the antenna became negative relative to the base.

EAGs elicited by the odor of a foreign ant are shown in the middle record of Figure 21. Sham tests on the latter half of the record were negative.

The relatively crude methods of stimulus application precluded a careful analysis of the nature of the odor-induced electroantennogram, but two generalizations emerged from the study and are illustrated in the upper and middle records of Figure 21. (1) All responses were monophasic and decayed to the baseline relatively slowly, regardless of stimulus duration. (2) Each stimulus usually diminished the responsiveness of the antenna to succeeding stimuli, but maximum responsiveness was regained after an interstimulus interval of one to several seconds.

Beta-Induced EAG

Four of eight isolated antennae gave an EAG to beta stimuli. The bottom record in Figure 21 shows typical responses to a train of beta flashes of various durations, delivered at a dose rate of approximately 2.2 rads/sec. When compared to odor-induced EAGs, beta responses were of opposite polarity, and response peaks were

somewhat sharper, with the decay of each deflection to the baseline

beginning immediately, irrespective of stimulus duration. There

also appeared to be a slight "off" response of opposite polarity upon

cessation of some of the beta stimuli.

The decrease in amplitude of successive responses was not

pronounced, nor irreversible. After a short rest, the antenna re-

sponded maximally to additional beta stimuli, as well as to adequate

olfactory stimuli.

Amplitude of response was noted to vary directly with dose

rate, although I did not attempt to quantify this relationship. Figure

22 shows a series of small EAGs to beta stimuli with the source held

and waved approximately four centimeters from the antenna (380

mrads/sec; see Table 3), followed by an illustration that 150 mrads/

sec failed to elicit measurable EAG responses, regardless of the

duration of exposure. Similar results were obtained with one other

antenna. Thus the threshold dose rate range required to elicit an

EAG was between 150 and 380 mrads/sec.

These data offer at least qualitative support for the evidence

derived from studies of behavior that olfactory (and perhaps other)

receptors on the antennal flagellum detect a stimulus that leads to

prompt behavioral reactions of carpenter ants to ionizing radiation.
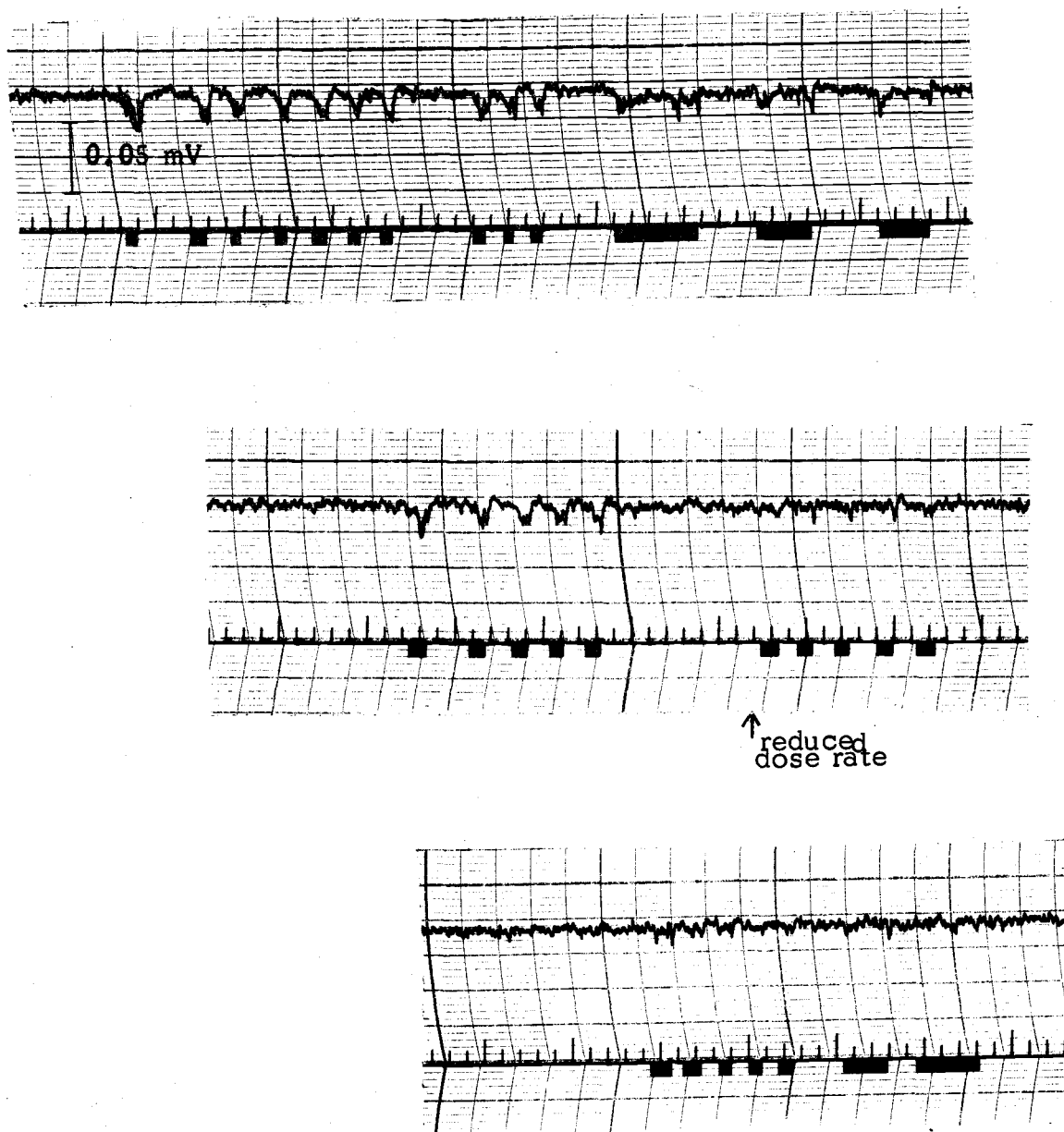
Figure 22. A continuous record of EAGs to flashes of beta radiation
delivered at 380 mrads/sec, followed by the failure of 150
mrads/sec to elicit pronounced EAG s, regardless of flash
duration. Tracings as in Figure 21.

Behavior Responses to Prolonged and Repetitive Exposures

Some observations made during the study of prompt reactions

suggested that ionizing radiation was acting as a noxious stimulus

for ants. (1) There was no waning of prompt reactions, especially

rapid running behavior, during irradiations at high exposure rates.

(2) Running activity resumed after a transient "off" response, some-

times approaching or equaling the strength of running exhibited dur-

ing exposure (Figure 11). Radiation had obviously induced a state of

excitement that persisted well beyond the period of exposure. In one

experiment, it was incidentally observed that the post-exposure run-

ning response lasted longer with each successive one-minute expo-

sure to 80 R/sec in a repeated exposure design. This suggested that

a cumulative, dose-dependent physiologic imbalance was being pro-

duced, and prompted me to explore further the aversive or noxious

properties of x-rays on ant behavior, and to determine the behavior

associated with the lethality response of ants to ionizing radiation.

Studies on aversion are presented below; lethality will be dealt with

in a later section.

Animals Exposed and Tested in the Fall:
Escape and Place-Avoidance Studies

If x-irradiation was in fact a noxious experience, ants provided

with the opportunity to do so might escape and avoid the beam during exposure. It should also be possible to teach (condition) them to avoid cues associated with the noxious stimulus, such as the place of exposure.

Two-compartment, free-choice chambers were employed to test these hypotheses. Four groups (n = 14/group) were habituated to the free-choice chambers. Analysis of residence preference during this time indicated a strong tendency to reside in the rough-bottomed compartment, and the pre-exposure preference score of each group for that compartment ranged from 0.8 to 1.0. With the smooth-floored compartment shielded, each group was irradiated while in the rough-floored compartment for five minutes, at the rate of 0, 20, 40, and 80 R/sec. Most members of each group except the controls (0 R/sec) gradually collected in the shielded compartment during exposure. After exposure, avoidance of the place of exposure could be observed (Figure 23). By 60 minutes post-exposure, most ants in each group were still avoiding the place of exposure, but by the following morning each group had resumed its pre-exposure preference for the rough-bottomed compartment that had been exposed to x-rays. High exposure rates of ionizing radiation appeared to constitute a noxious stimulus for these ants, but neither the rate of escape nor the duration of avoidance was strongly dependent on exposure rate or accumulated dose (Figure 23).
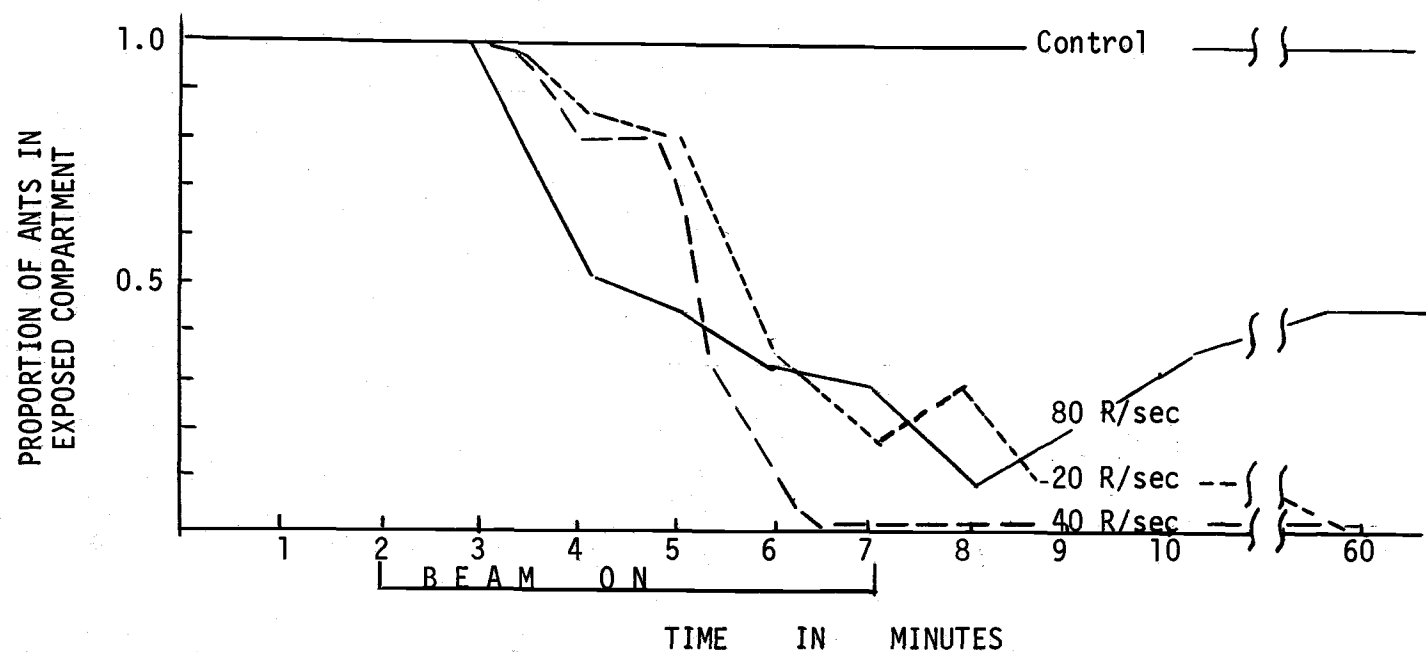
Figure 23. Escape from the exposed compartment during irradiation, and subsequent place-avoidance response, of groups exposed at three different exposure rates for five minutes each (n=14/group). C. laevigatus, October.

To study the duration of conditioned place avoidance as a function of reinforcement by repeated exposures to the US, two new experimental groups were exposed to 80 R/sec according to the following schedule (a third group served as a sham-irradiated control): The preferred compartment of each group was exposed for five minutes, during which time most ants escaped from and avoided the beam. Group 1 was then observed for an hour, and returned to the ant room to be exposed again at a later time. The unshielded compartment of group 2 continued to receive short bursts of x-rays as subjects returned to that compartment following termination of the initial exposure.

The results of this phase of the experiment are presented in Figure 24. Members of group 1 returned to the exposed compartment relatively quickly (half had returned by one hour after irradiation), but each short burst of x-rays drove the ant(s) in group 2 back into the shielded chamber. After the final long burst, at 45-50 minutes after the initial exposure, this group avoided the place of exposure not only to 100 minutes (Figure 24) but for nearly two days thereafter.

After ants in both groups had resumed their initial preference for the rough-floored compartment, each group was given another five minute exposure. As before, members of group 2 then continued to receive short bursts of x-rays as they re-entered the exposed
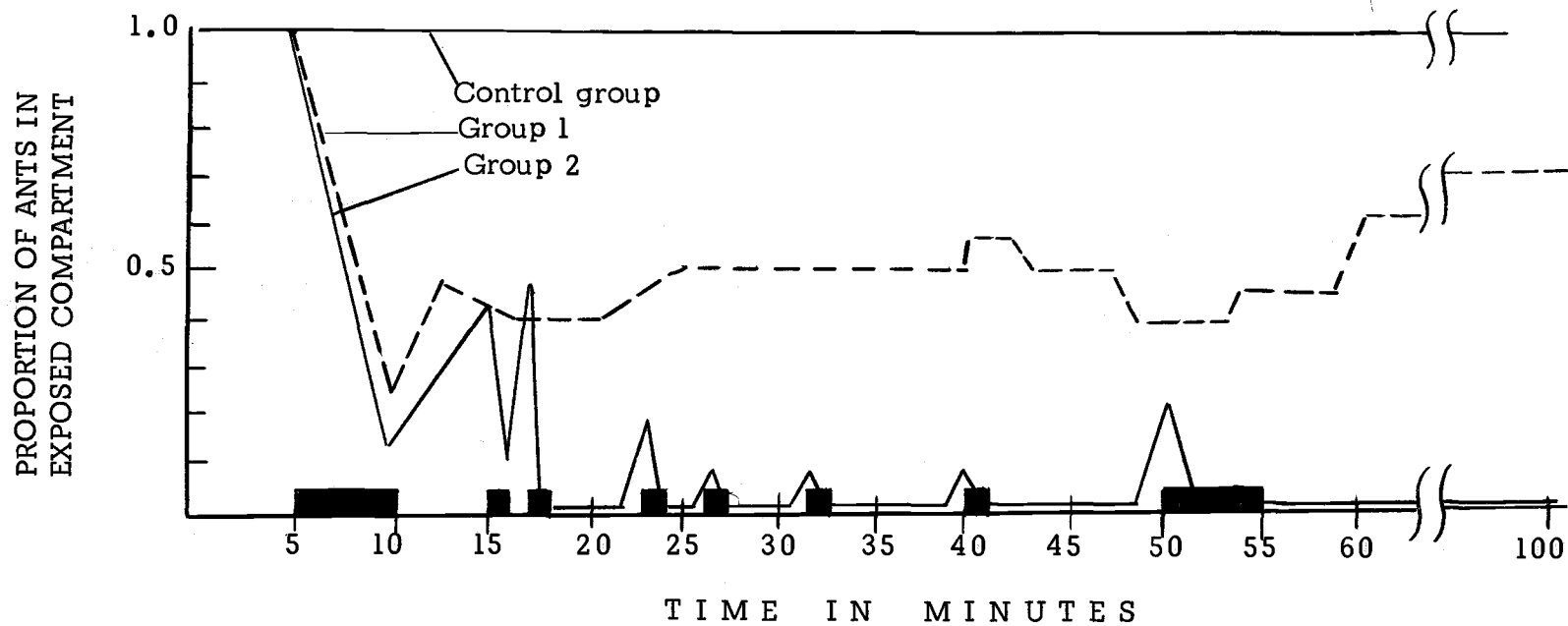
Figure 24. Escape and conditioned avoidance by two groups initially exposed to 80 R/sec for five min. C. laevigatus, November. Members of group 2 continued to receive short bursts of x-rays (dark bars along time axis) as they returned to the unshielded compartment. N=10-14.

compartment. The long-term results of this entire experiment can

be seen in Figure 25. Note that members of group 1 moved back and

forth between compartments for several days following the second

exposure, then came to rest mainly in the compartment that was not

exposed to x-rays. Members of group 2 exhibited complete avoidance

of the exposed compartment after only two short bursts (bursts not

shown in Figure), whereas seven bursts were required after their

initial exposure (Figure 24). Further, the conditioned avoidance

response of this group persisted for at least 12 days, at which time

the experiment was terminated due to deaths of some subjects in the

control as well as experimental groups.

<div align="center">

Seasonal Differences in Responsiveness:
Attraction to the Beam

</div>

Initial studies on conditioned avoidance behavior (above) were

done in the fall of the year (October - November). All subsequent

experiments in free-choice chambers were done in the spring (March-

May), during which time I attempted to duplicate and extend the work

just reported. In late March, 20 ants were collected as they emerged

from hibernation and were distributed into two free-choice chambers.

During the three-day habituation period, these ants failed to exhibit

a strong preference for either compartment. With about half of the

subjects in each side of the chamber, I shielded the smooth-bottomed
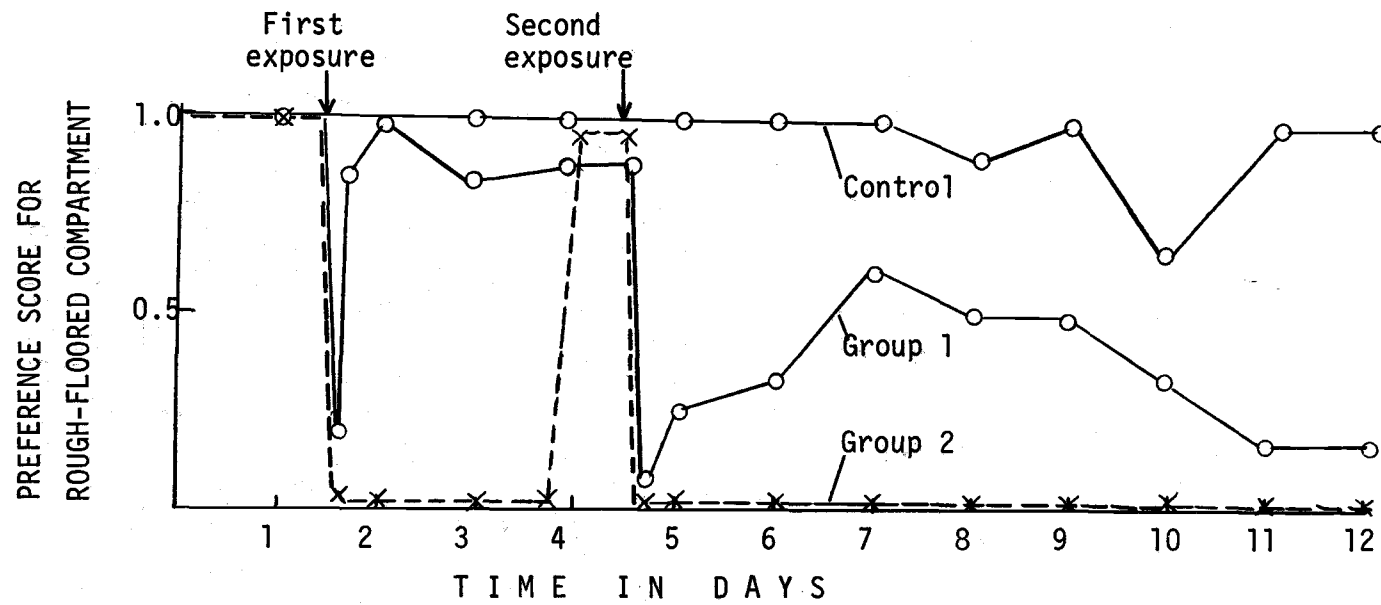
Figure 25. Long-term conditioned-avoidance responses resulting from two different successive exposure regimes. Details of the responses given to the first exposure are shown in Figure 24.

side and exposed the rough-floored compartment to 20 R/sec for five minutes. The control group was sham exposed for the same length of time. Rather than escaping, the ants being irradiated initially ran back and forth between compartments, causing the ants in the shielded side to become active, and within 90 seconds all 10 ants had collected in the exposed compartment. There they remained, very active, for the duration of the exposure. A pronounced "off" response was given by most subjects upon termination of exposure, then a high level of activity was resumed, with ants running back and forth between compartments as if in a confused state. Gradually they quieted down, about half in each compartment, as prior to exposure.

Subsequent irradiation of the unshielded compartment at exposure rates of 10, 40, and 80 R/sec resulted in similar behavior: movement into the exposed compartment during exposure, and dispersal throughout the chamber between exposures. Thus, during irradiation, ants exhibited a marked attraction to (rather than escaping from) the beam; following exposure there were no indications of the development of a conditioned place-avoidance response.

I then prepared two new groups (n = 10/group) and repeated the regime that had resulted in the long-term conditioned place avoidance in the fall (Figure 24). One group served as controls, and the experimental group received 80 R/sec for five minutes, followed by reinforcing bursts of irradiation comparable to those given in the fall.

The results were that each time the beam was turned on most or all

ants collected in the exposed compartment rather than escaping from

it. Between exposures, they ran back and forth between compart-

ments as if searching for the beam of x-rays. These results in

April are the opposite of behavioral responses given by the same

species, maintained and exposed under identical conditions, during

November, as can be seen in Figure 26.

Further experimentation revealed that both northern and giant

carpenter ants behaved identically, exhibiting attraction to the beam

in the springtime.

Other seasonal differences in responsiveness soon became

apparent. Prompt responses were found to be faster and stronger in

the spring than in the fall. For example, latency of the antennal-

waving response was several times faster in April than in October,

at each of several exposure rates (Figure 27). Additionally, captive

ants appeared to maintain a somewhat higher level of activity in the

spring, and tended to remain dispersed throughout a free-choice

chamber rather than clustering together in one compartment, as was

typical of ants tested in the fall (compare the control groups in

Figure 26).

Were X-Rays a Noxious Stimulus?

Ants tested in the spring responded as though they were
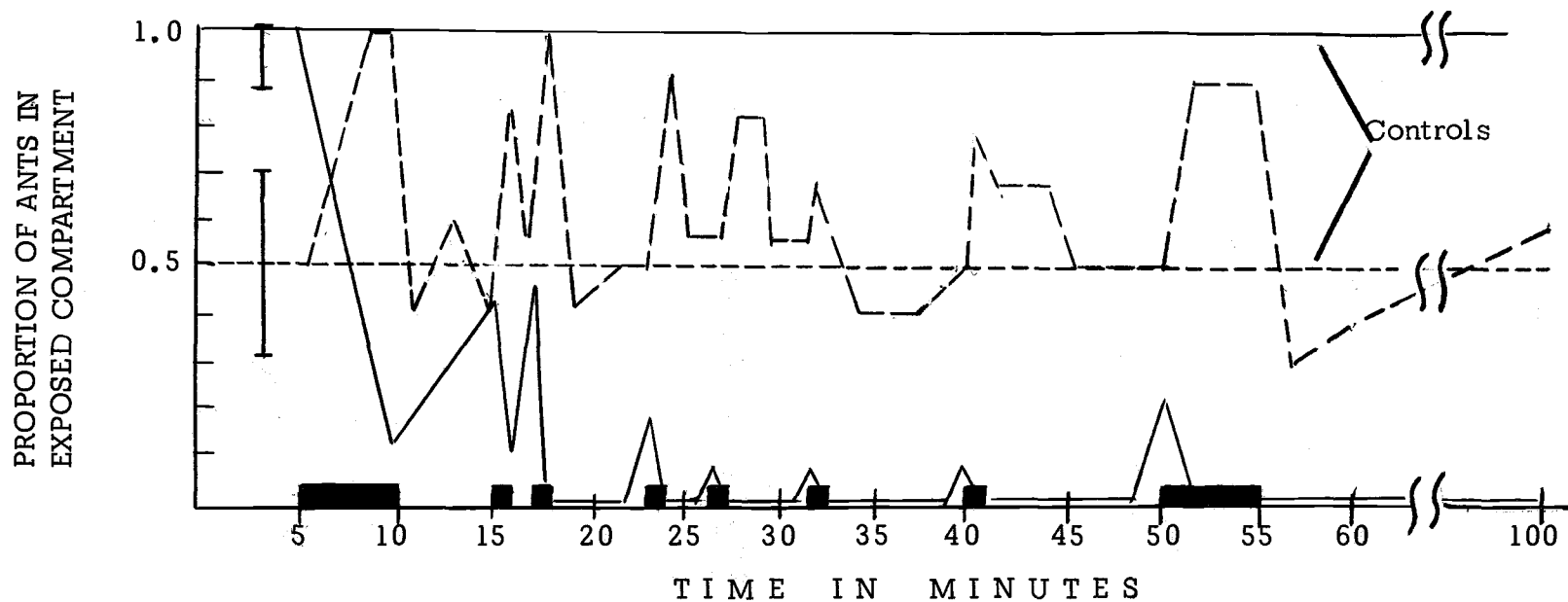
Figure 26. Movements within the free-choice chamber of C. laevigatus exposed to bursts of 80 R/sec (bars along time axis) in November (solid lines) and in May (dashed lines). Sham-exposed controls strongly prefered the exposed compartment in November, but exhibited no preference at all in May. Error bars show range for control groups.
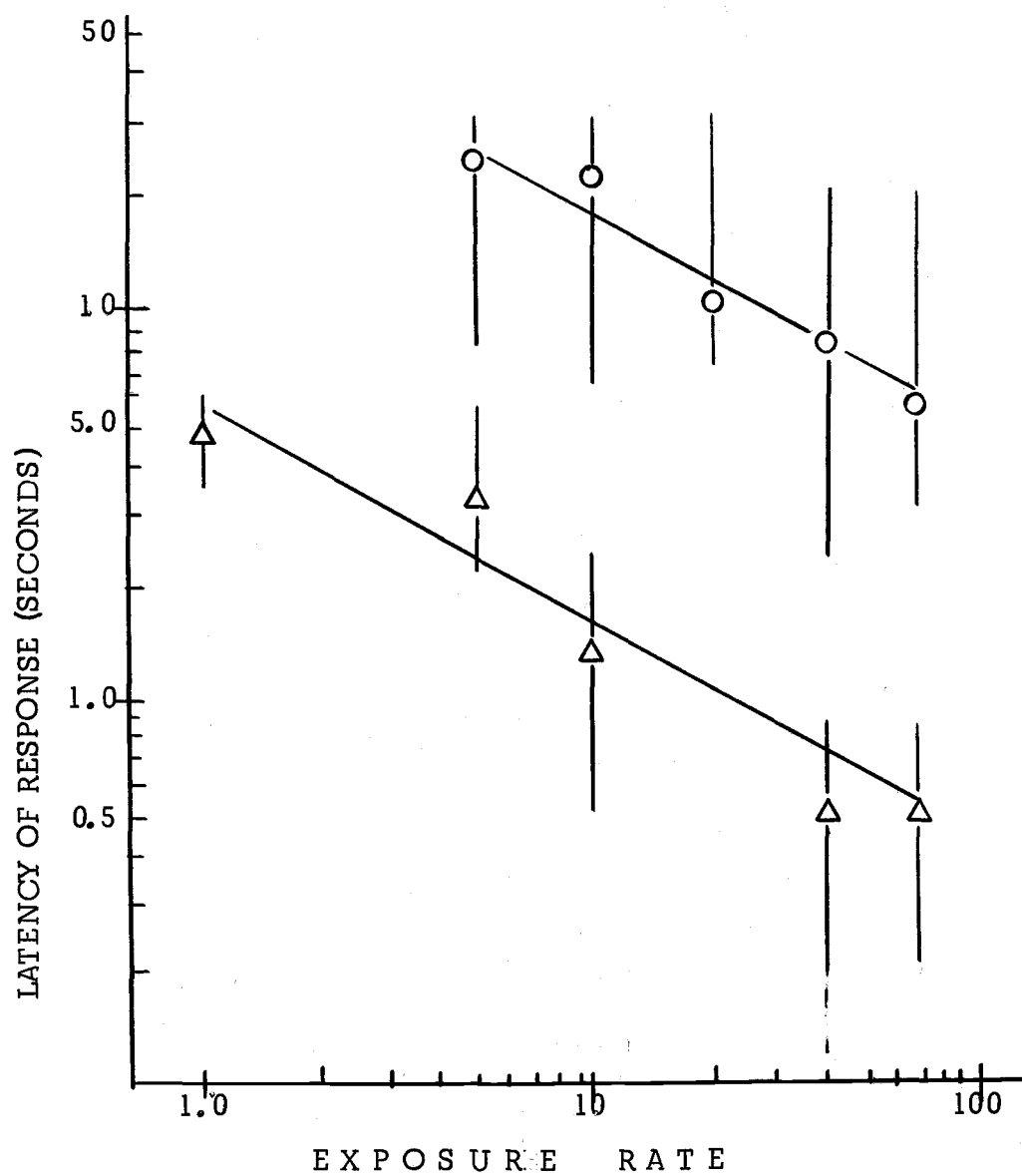
Figure 27. Comparison of the latency of antennal responses
of giant carpenter ants exposed in May (△) and in
November (O). Each point = mean response time
for each group (n=5-14); bars show range.

physiologically different from ants in the fall. This led to the formulation of two alternative hypotheses: (1) X-rays led to aversive behavior only at certain times of the year. (2) Assuming that the apparent attraction to the beam exhibited in the spring was a transient artifact (to be discussed), x-rays were noxious at all times, but ants tested during the spring were unable to learn to associate the radiation stimulus with the place of exposure. It was possible that the stimulus difference between compartments lacked the necessary distinction for ants tested in the spring to learn to associate one compartment with the noxious stimulus. Another possibility was that the stimulus characteristics of radiation were not appropriate for ants tested in the spring.

Accordingly, I modified several of the chambers so as to enhance the ease of discrimination between the compartments (see Methods). Then sixty ants were equally divided into six groups, habituated in the new chambers, then exposed to the same total dose on each of 10 consecutive days. Each group received a different exposure rate and duration, according to the schedule in Table 4, so as to assess the effects of different exposure conditions.

During the habituation period, no group demonstrated a preference for one compartment over the other, so I decided to shield the smooth-bottomed compartment during all exposures. All exposures were viewed on closed-circuit TV, and most were recorded on tape

for subsequent analysis.

Table 4. Conditions of Exposure for Six Groups Exposed Once
on Each of 10 Consecutive Days in the Modified Free-
Choice Chambers.

Group	Stimulus strength (R/sec)	Stimulus duration (min)	Total daily exposure (kR)
1 (controls)	0	several min.	0
2	1	60	6
3	5	20	6
4	10	10	6
5	20	5	6
6	40	2.5	6

As in previous experiments in the spring, groups exposed to

20 and to 40 R/sec became very active, remaining in or moving into

the unshielded compartment during each exposure. Occasionally,

an ant would leave and remain out of the beam. The more typical

response on moving into the shielded compartment was to immedi-

ately "test" the environment by stopping, waving and cleaning antenna

(as in a usual "off" response), then turning around and moving quickly

back into, and remaining active within, the unshielded compartment.

On termination of exposure, activity waned for a few seconds, then

increased, with subjects running back and forth between both ends of

the chamber, as if in a confused state.

If, on one day, the 40 R/sec group appeared to be more strongly attracted to the beam than the 20 R/sec group, the opposite was generally true on the next day. For each group, attraction was just as pronounced during the tenth exposure as during the first, and between exposures neither group gave evidence of a sustained preference for, nor avoidance of, either compartment.

The groups receiving 10 and 5 R/sec also exhibited attraction, but to a lesser extent than ants irradiated at higher exposure rates. For example, ants that were not in the exposed compartment at the onset of irradiation often remained relatively inactive in the shielded compartment throughout the 10 or 20 minute exposure. Ants that did experience the beam displayed varying degrees of excitement. Most remained active in the beam, but some, especially in the 5 R/sec group, settled back down during exposure, either in the beam or after having moved into the shielded region.

Exposure of the group receiving only 1 R/sec was discontinued after the first trial, because even though exposure lasted for one hour, only six of ten ants experienced the beam; the other four remained resting in the shielded compartment. Ants in the radiation field gave distinct evidence of detection, by antennal waving and slow running. These subjects, however, either habituated to the beam, or moved slowly around throughout the chamber.

The results of this experiment on ants tested in the spring

indicates that detection of radiation occurred, but without any sign of subsequent behavioral conditioning. This failure to condition occurred despite the accentuation of spatial cues and a wide spectrum of exposure conditions.

Most members of the 20 and 40 R/sec groups collected in the irradiated compartments during most of each exposure. They probably received, on the average, in excess of 40 kR during the 10 consecutive exposures (6 kR per exposure to the unshielded region). This cumulative exposure to massive amounts of radiation did not appear to affect the behavior of the subjects. To answer the inevitable question of whether ants in the springtime would escape from the beam prior to radiation-induced incapacitation or death, I exposed two new groups (n = 10/group) to a continuous, uninterrupted x-ray beam at 80 R/sec and 20 R/sec, respectively, for a period in excess of 40 minutes.

The 80 R/sec group became highly active immediately, running within the unshielded region, or back and forth between compartments, at great speed. Initially, some ants stopped after entering the shielded compartment and gave typical "off" responses, then returned quickly to the exposed region. After approximately 15 minutes of exposure, ants tended to cease giving "off" responses on entering the shielded side, but the very high level of activity persisted, regardless of which compartment an ant was in. There was never

any indication of avoidance of the beam for any long period; rather,

ants appeared to be losing the ability to discriminate between the

environments of the exposed and the shielded portions of the chamber.

Between 22 and 30 minutes of exposure, several subjects ex-

hibited signs of ataxia. They began to fall over on their backs and

were unable to right themselves quickly. Their hind limbs appeared

to be partially paralyzed. At 25 minutes, the first ant dropped over,

unable to regain an upright posture and, by 39 min, all ants were on

their sides or backs, with three prostrate in the shielded portion and

seven in the exposed portion of the chamber. Each ant was highly

active until it suddenly became immobilized, but none exhibited any

tendency to escape from nor to avoid the beam. (The lethality re-

sponse will be analyzed in a later section).

Behavioral reactions of the group exposed at 20 R/sec were

similar to those seen in the 80 R/sec group, but developed at later

times during exposure. The intensity of running activity was less

at first, but gradually increased, and after several minutes most

subjects were running at rates which appeared to approach the upper

limits of their physical ability. Despite a great deal of individual

variation in this parameter, by 45 minutes most subjects had ceased

to exhibit attraction to the beam. That is, on moving into the

shielded compartment, they no longer gave "off" responses followed

by a prompt return to the place of exposure. Rapid running back and

forth continued, however, with no clear evidence of avoidance of the radiation field except by one of 10 subjects.

By 80 minutes there was evidence of ataxia, and the first two ants became immobilized, in the exposed compartment, at 97 and 98 min. At 110 min, there was no hint of sustained avoidance behavior, even though several ants were still very actively running back and forth. Exposure was terminated at this point. Subjects not yet incapacitated remained highly active for several hours after exposure.

According to the criteria I had established as necessary indicators of aversion, these results have to be taken as evidence that ionizing radiation was not a noxious stimulus to ants in the spring of the year. The results did show that : (1) Continuous, long-term exposure induced an intense, persistent state of excitement. (2) After the accumulation of approximately 50 to 60 kR, ants ceased to give evidence of discrimination between the radiation field and the environment of the shielded compartment. (3) Following exposure to about 100 kR, behavioral manifestations of direct radiation damage appeared (to be discussed later). It is not known to what extent (1) and (2) above were due to radiation damage.

Mechanisms of Action

There was no evidence to suggest that the behavior patterns which developed during relatively short exposures - radiation

avoidance in the fall, and attraction in the spring - were due to radiation damage. Thus, the question of mechanisms by which ionizing radiation effected these behavioral responses is relevant to this study.

It was possible that the x-ray stimulus might have caused the elaboration of the same or different pheromones, which attracted the ants at one time of year, but repelled them at another time (Wilson, 1965). To test this hypothesis, 10 ants were placed in 10 separate free-choice chambers, on the assumption that any contribution by other ants to a response would not be exhibited by individuals exposed separately. After habituation, the compartment in which each ant was resting was exposed to 20 R/sec for five minutes (the other compartment was shielded). For 80 percent of the subjects, the fraction of the five-minute exposure period spent in the beam ranged from 0.62 to 0.97 (average = 0.77). This, again, illustrated the attraction to the place of exposure in spring-exposed ants. There was no discernable difference in any aspect of the behavior of ants exposed individually, and ants which had been exposed in groups in the previous studies. It therefore seems very unlikely that the response could have been due to, or strongly influenced by, the release of pheromones, or by any other type of social facilitation.

Another possible means of indirect attraction involved the visual system. All exposures had been made in a well-lighted x-ray room, to minimize the possibility of a phosphene effect. Despite this

precaution, it is conceivable that high exposure rates might have been sufficient to stimulate photosensitive components of the light-adapted eye. I therefore ophthalmectomized and exposed two groups (n = 5/group) to 10 R/sec and 40 R/sec, respectively, for five minutes each. Both groups showed the same attraction to the beam as that described above for normal subjects exposed at comparable rates. The only difference was the absence of a conspicuous "off" response by ophthalmectomized subjects on termination of exposure. The migration of ants into the beam in the spring was thus unrelated to visual stimulation.

A final experiment was done to determine to what extent the behavioral responses to prolonged exposures depended on sensory mechanisms known to be intimately involved in prompt behavioral reactions of carpenter ants, namely antennal receptors. Two groups were antennectomized, placed in partially-shielded free-choice chambers, and exposed for five minutes to 10 R/sec and 40 R/sec, respectively.

Some of the antennectomized ants responded to prolonged exposure, although the latency of response was relatively long (Table 5). (Recall that during the study of prompt responses, all antennectomized ants were exposed only to short, 30 second bursts of x-rays at 10 R/sec).

Table 5. Responsiveness of Antennectomized Ants to Prolonged (Five Minute) Exposures in Free-Choice Chambers.

Exposure rate (R/sec)	Speed of running response	Fraction responding	Latency of response (average)	(range)
10	moderate	3/6	92 sec	(70 - 121)
40	fast	5/5	40 sec	(11 - 55)

Half of the ants that were in the unshielded compartment during exposure to 10 R/sec became active during the five-minute irradiation. They remained in the beam, moving around slowly. The group exposed to 40 R/sec responded much faster and stronger. One ant became highly active in 11 seconds, but the average latency of response was 40 sec (Table 5). All ants were active within one minute, and by three minutes they were running intensively, in a manner similar to the behavior pattern of normal ants. Midway through the exposure two ants began fighting, and locked together in a death grip, but at least two of the others exhibited attraction to the beam. That is, on leaving the beam they would either stop running and attempt to clean (nonexistent) antenna, then return to the unshielded compartment, or quickly turn around and move back in without stopping. At the end of exposure a strong, transient "off" response was displayed.

The results of this experiment revealed that antennal receptors are not essential in the response of attraction to the beam. It also

became apparent that the "off" response, and the rapid running be-

havior during and after prolonged exposures at high rates, were

independent of the antennal mechanisms responsible for prompt be-

havioral reactions to x-rays.

Lethality Syndromes

In September, 56 worker ants were distributed into five experi-

mental and one control groups (n = 8-10/group), placed in small,

single-compartment chambers (see Figure 4), and exposed at 25

R/sec to 25, 50, 100, 125, and 150 kR, respectively. Figure 28 de-

picts the weekly distribution of post-exposure mortality at each expo-

sure level over the first 25 weeks of observation. Note that the tem-

poral distribution of deaths depended strongly on total exposure. All

animals receiving 50 kR or more died by or before the 20th week

after exposure. By contrast, at 27 weeks, 80 percent of the 25 kR

group, and 90 percent of the controls, were alive and in apparently

good health.

The mortality data were subjected to quantal response analysis

(see Methods) for calculation of the dose-response relationship for

mortality within 30 days of exposure. The results of this analysis

are plotted in Figure 29. The calculated LD $50/30_{\text{days}}$ was 101 kR,

with 95 percent confidence limits of 82 and 116 kR. Similar analysis

for mortality within a 60-day period resulted in an LD $50/60_{\text{days}}$ of
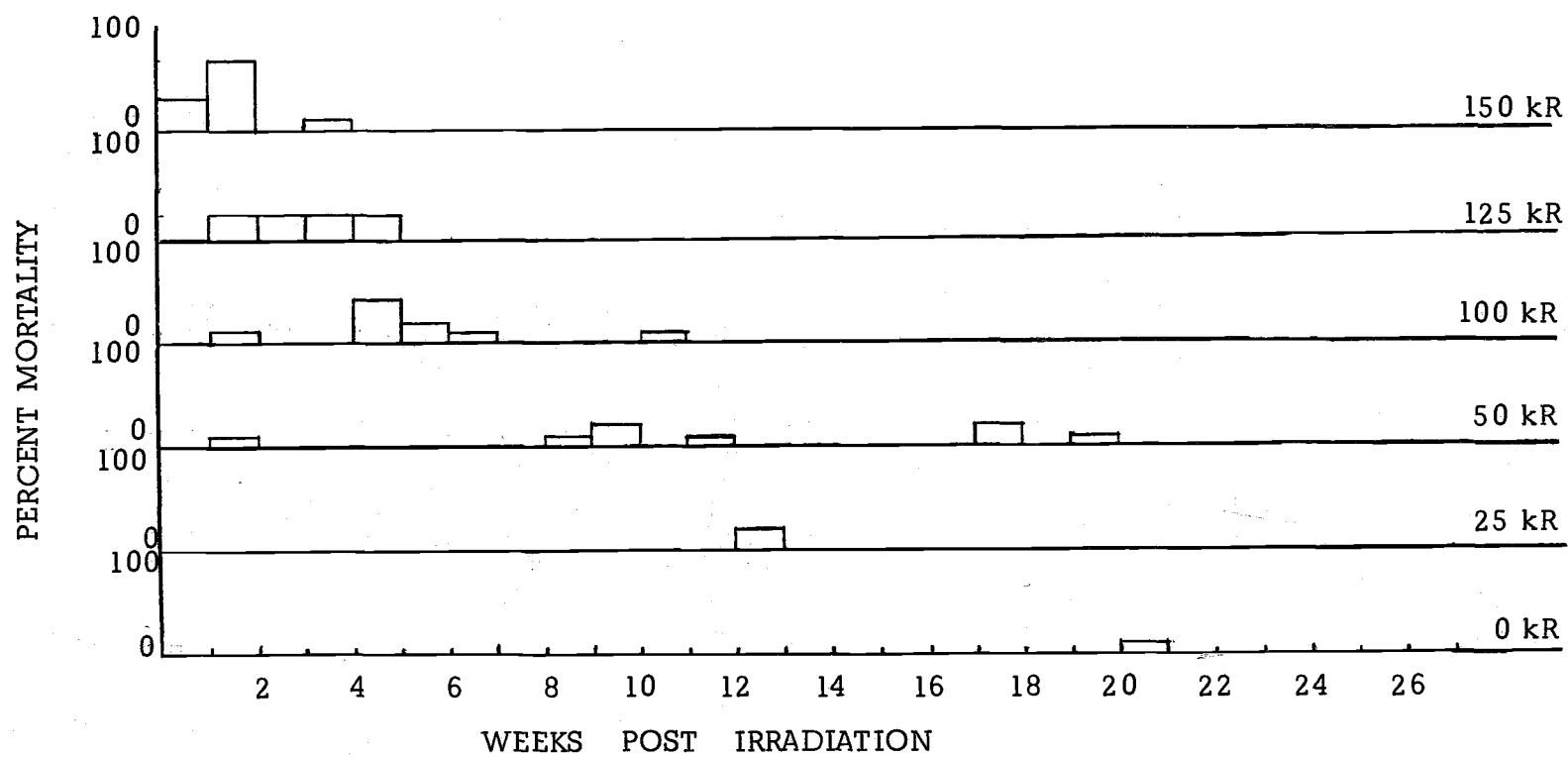
Figure 28. Distribution of deaths (weekly mortality) following irradiation at different total exposures.
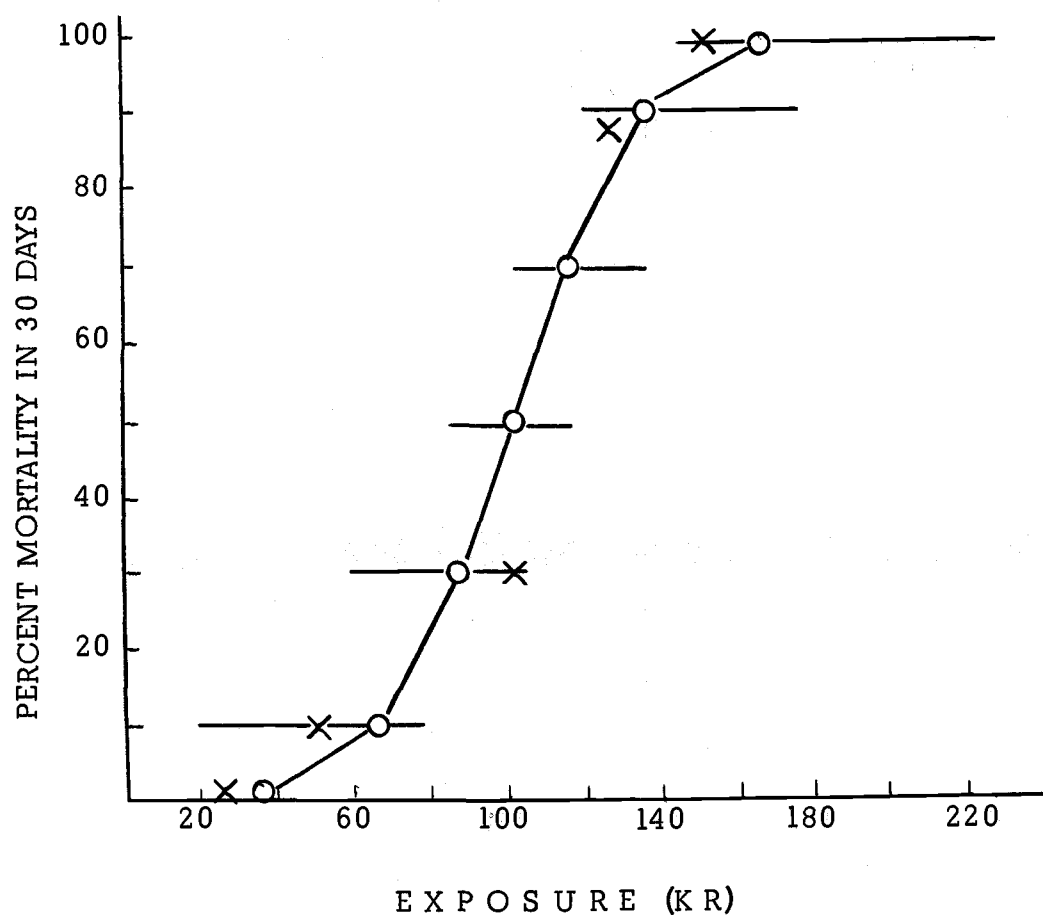
Figure 29. The exposure-response curve derived from quantal response analysis of the mortality data. Bars show 0.5 confidence limits, and symbol X indicates raw data point.

66 kR, with confidence limits of 46 and 84 kR.

During the 27-week study, the behavior of members of the 25 kR group was not different from the behavior of control subjects, except for the high degree of excitement (rapid running) shown during, and for several hours after, exposure to x-rays. However, at exposure levels of 50 kR or more, animals manifested two distinct behavioral syndromes that depended on the amount of exposure. The first was transient, and developed during and immediately following exposure; the other was a terminal syndrome associated with morbidity, and occurred just prior to death. Each syndrome will be described in turn.

During irradiation, with the accumulation of between 82 and 99 kR, ants appeared to become stunned. While running rapidly, subjects began to lose motor control of their hind legs, and soon lapsed into a comatose state. They appeared to be dead but for the occurrence of slight twitchings or tremors of the extremities. The duration of this comatose state increased as the total exposure was increased (Table 6).

During recovery from the apparent coma, the legs of all subjects began to twitch and jerk, a condition which persisted for several days thereafter. The severity of this condition and the rate of recovery were proportional to the total exposure. Ants receiving 150 kR failed to fully recover from leg twitching prior to death (Table 6).

Table 6.   The Duration of Behavioral Manifestations of the Early
Radiation Syndrome.   Values Represent the Approximate
Recovery Times for Half of the Subjects in Each Experi-
mental Group.

| Behavior | Group | | | |
	50 kR	100 kR	125 kR	150 kR
Comatose state	--	30 min	12 hours	30 hours
Leg twitching	3 days	5 days	10 days	(died first)

The terminal, morbidity syndrome involved a loss of postural

control and a general discoordination of limb movements.   This syn-

drome preceded death by a few hours to a few days, depending on

dose.   The severity and duration of the syndrome decreased with de-

creasing dose.   Following exposure to 150 kR, one subject died on

day 4, without recovering from the comatose state.   Others had a

difficult time righting themselves, and moved shakily, in short, jerky

steps.   However, when I rapped lightly on the exposure chamber, sub-

jects demonstrated the ability to suppress tremors immediately, and

to remain motionless for several seconds.   The terminal syndrome

that developed between days 9-14 in this group included the following

major features: loss of ability to perform simple, innate motor

patterns such as cleaning of antenna; walking with head inverted be-

tween front legs, exhibiting periodic spasms, and displaying indif-

ference to all but the strongest mechanical stimuli.   Just prior to

death, ants were observed to lie on their sides or backs most of the time.

In general, this pattern of lethal injury was less pronounced and developed later in time for the 125 and 100 kR groups, respectively. The terminal syndrome was not apparent preceding the widely-distributed deaths (Figure 28) in the 50 kR group.

Based on the reactions displayed, both syndromes would seem to have involved either direct or indirect damage to the nerve and muscle systems.

DISCUSSION

An initial objective of this study was to seek out and character-
ize prompt behavioral responses given by carpenter ants to bursts of
ionizing radiation and to uncover the potential mechanisms through
which detection could occur. In this manner, I hoped to extend the
body of knowledge on a topic that has been developed almost entirely
from intensive studies on selected vertebrate species.

Immediate, transient behavioral responses - antennal waving
and running behavior - were found to occur at exposure rates as low
as 0.05 R/sec. Characteristics of each response, such as latency,
strength, duration, and percent subjects responding, were strongly
dose-rate dependent (Figures 9-11). This suggested that excitable
tissues were stimulated by something inherent in or accompanying
exposure to x-rays.

Any artifactual cue would have had to vary in intensity with the
exposure rate. Possible candidates included sound of the x-ray
machine and radiation-induced fluorescence in the walls of the expo-
sure chambers. Subjects did not respond when the chamber was
shielded from x-rays but not from sound, and exposures were made
in a well-lighted room to mask the potential effects of fluorescence.
Another possible stimulus was atmospheric by-products of ionization,
such as ozone or oxides of nitrogen. Little work has been done on this

problem, since it is difficult to measure the concentration and duration of radiolytic by-products resulting from irradiation of air (Levy, 1967). However, ants with shellaced antennae exhibited detection on exposure suggesting that the effect was mediated within or on the immediate surface of antennal receptors. Thus none of these concomitants of exposure appeared to be responsible for prompt behavioral reactions to x-ray stimuli.

Two previous studies on arthropods have attempted to identify the physiological mechanisms by which x-ray stimuli elicit prompt behavioral responses. Terwilliger and Levy (1964) discovered that crabs (Uca) ceased to give an "off" response when their eyes had been extirpated. A more extensive series of investigations (Smith, et al, 1963; Smith and Kimeldorf, 1964) revealed that various species of moths gave prompt motor responses to ionizing radiation if they were exposed in a dark-adapted state; in a well-illuminated room no responses were observed to occur. These investigators used behavioral and electrophysiological techniques to show conclusively that the motor responses of moths were mediated by the dark-adapted visual system, and occurred promptly at exposure rates as low as 10 mR/sec.

In contrast to moths, I found that light-adapted carpenter ants gave prompt motor responses upon exposure to x-rays, although the threshold exposure rate was somewhat higher (50 mR/sec.) Bilateral

ophthalmectomy did not prevent the initial responses. However, the pronounced "off" responses exhibited by normal subjects on termination of long exposures at high rates were not displayed by ants whose eyes had been removed. Thus, visual detection might have played a part in the transient cessation of activity as the beam went off.

Isolated ant eyes gave strong, reversible bioelectric responses to x-rays, the characteristics of which were very similar to those elicited by light stimuli, suggesting similar mechanisms of action of light and x-rays on the photosensitive pigment molecules in the omatidia.

ERG responses were also given to stimuli of beta radiation, at dose rates as low as 62 mrads/sec, with flash durations of 1/20 sec. Thus, a threshold absorbed dose per flash of only 3. 1 mrads was an effective stimulus for the eye. This is somewhat higher than the 0. 25 mR to elicit an ERG response from the eye of the moth, Pseudaletia unipuncta, reported by Smith and Kimeldorf (1964).

The irreversible decline in responsiveness to a series of beta stimuli has been observed in the eyes of other arthropods (Jordan, 1970), but is not understood. This permanent decrease differed in several ways from the highly transient decrease to a train of rapid stimuli of light or x-rays (Figure 20), and did not inactivate the photoreceptive mechanism that was responsive to light (Figure 19). Thus beta-induced ERGs appeared to involve a different or an

additional mode of action than that underlying the responses to light and to x-rays.

Results of the series of experiments on antennectomized ants leave little doubt that, under the conditions of this study, the principle route by which x-ray stimuli evoked immediate, transient behavior changes in carpenter ants was via receptors in some or all of the nine distal segments of the antennal flagella (Figure 13). Evidence was also derived, from behavioral and electrophysiological experiments, which indicated that the receptor neurons involved were olfactory neurons.

Electroantennograms were elicited from isolated antenna by several olfactory stimuli and by ionizing radiation (Figure 21). An understanding of the differences between odor-induced EAGs and those given upon exposure to pulses of radiation must await further study. Detrich Schneider, the only worker who has extensively studied the EAG response, reported that the form of the EAG depends on several unknown factors; different odors, and even the same odor at different times, have resulted in markedly different electroantennograms in silkworm moths, Bombyx (Schneider, 1957b, 1962).

The threshold dose rate to elicit an EAG from half of the antennae studied was between 150 and 380 mrads/sec (Figure 22). Within this exposure range (at 200 mR/sec) about half of the ants also gave prompt behavioral evidence of detection (Figure 9), but the

response time was considerably longer for the antennal-waving response than for the appearance of an EAG. Strict quantitative comparisons of these behavioral and electrophysiological responses are not possible, since different kinds of ionizing radiation were employed as stimuli. Also, the precise relationship between an electrical response and the motor system involved in the behavior is not known as yet.

No previous study has implicated olfactory (or any antennal) receptors in the prompt detection of ionizing radiation by arthropods. As mentioned earlier, olfactory receptors are known to participate in the prompt detection of x-rays by mammals like rats and monkeys (Smith, 1971). Threshold exposure rates for bioelectric responses from the olfactory bulbs of rats (Cooper, 1968), and for behavioral evidence of detection in rats (Garcia, et al. 1964) and in monkeys (Smith and Tucker, 1969) range from three to 50 mR/sec. These rates are lower than the threshold exposure rates found necessary to elicit comparable response in ants (above), but the important point is that the olfactory system now appears to be intimately involved in prompt responses to ionizing radiation in animals as diverse as ants and primates.

Under appropriate conditions of dark adaption, the visual and the olfactory systems of mammals exhibit roughly equal sensitivities to ionizing radiation (Smith, 1971). In my study of ants, antennal

receptors were much more important than the visual system in mediating prompt behavioral responses. The bioelectric responses of the isolated eye were more sensitive, however, than those elicited from the isolated antenna (threshold doses of 3 mrads vs. over 100 mrads, respectively). These differences might well have been due to technique. For example, I used extremely crude methods of delivering stimuli to the isolated antenna. This problem notwithstanding, it is now clear that animals spanning a wide phylogenetic range utilize visual and olfactory systems as the two primary routes of detection of ionizing radiation.

Another major objective of this study was to examine the aversive properties of x-rays for the behavior of ants. As mentioned in the Introduction, several excellent studies have established that mammals quickly learn to avoid cues (CS) that were previously associated with unconditioned stimuli of ionizing radiation. These studies have established that ionizing radiation is, by definition, a noxious stimulus for mammals (Kimeldorf and Hunt, 1965; Smith, 1971). No studies of this kind had been done with any invertebrate, but there were suggestions from the literature that ionizing radiation possessed noxious properties for animals like ants and sea urchins, in that these animals reportedly moved out of a beam of x-rays during exposure. For example, Hug (1960), using an unfiltered beam from a 50 keV x-ray machine reported that on irradiation of C. herculeanus

with 70 R/sec

> ...a lively disturbance ensued after 4 seconds. The insects
> show irregular, wild movements and more and more move in-
> to lead-protected half of the container. Should they again
> chance to approach the irradiated zone, a typical 'radio-
> phobic' reaction is observed. After 25 seconds, all insects
> have gathered in the unirradiated compartment; only a few
> still wander occasionally into the irradiated compartment.

This escape and avoidance behavior during exposure suggests

that ionizing radiation was noxious to ants, but no mention was made

about what the subjects did after exposure was terminated. A condi-

tioned place-avoidance response would have provided firm evidence

that radiation was noxious to ants.

The results of my preliminary studies with C. laevigatus in

October and November to some extent confirmed, and certainly ex-

tended, Hug's brief commentary. At exposure rates ranging from

20 to 80 R/sec, ants slowly escaped from and avoided the unshielded

compartment during exposure, and some subjects continued to avoid

the place of exposure for several hours (Figure 23). Another group,

driven out of the unshielded (preferred) compartment, and subse-

quently exposed to a series of short bursts ("punishments") each

time one or more ants returned, was conditioned to avoid the place of

exposure for 12 days following the final burst of x-rays (Figures 24,

25). The sham-exposed control group was critical here, in that by

continuing to exhibit strong fidelity to the "exposed", rough-floored

region, they illustrated that this compartment was, in fact, preferred.

In April, 1971 (three months prior to completion of the experi-

mental portion of this study) I attempted to repeat and refine the work

done in the fall with C. laevigitus, and to extend it to include G. her-

culeanus, the principle subject during all other phases of this investi-

gation. Both species not only failed to exhibit escape and avoidance,

they demonstrated attraction to the beam during exposure (Figure

26). The remainder of the study was devoted mainly to attempting to

understand something about this apparent seasonal reversal of respon-

siveness. The following points emerged: (1) Attraction to the place

of exposure was transient, e. g. it occurred only during exposure;

ants could not be conditioned to remain within (nor to avoid) the place

of exposure, regardless of the strength, number, or temporal dis-

tribution of exposures. (2) Attraction continued until subjects had

received a cumulative exposure of something like 60 to 70 kR, after

which they ceased to give evidence of discrimination between the

shielded and unshielded compartments, presumedly because of direct,

radiation-induced damage. (3) Within the limits of 1 - 40 R/sec,

attraction was less a function of total dose than of dose rate (Table

5 plus comments). (4) Movement into the beam during prolonged ex-

posure was shown to occur in the absence of visual or olfactory sys-

tems; systems that are known to be the major routes of detection

leading to immediate, transient behavioral responses.

Axenfeld (1897a) observed that a variety of arthropods moved

into an x-ray beam, unless they were blinded:

> If insects (Coleoptera, Diptera, Hymenoptera) or crustaceans
> (Porcellius) are taken in a box, half of which is made of wood,
> half of lead, and if this box is placed in the influence of
> Roentgen rays for a short time, the enclosed animals wander
> into the part of the box that is pervious to the rays; thereby
> one may be led to assume every possibility of a sense of sight,
> for blinded animals do not show this capacity.

These results were confirmed later by the same author (Axenfeld,

1897b). In my study, visual detection of x-rays was not responsible

for attraction since blinded ants behaved as did normal subjects.

Wilson (1965) reported that an alarm pheromone of the harvester

ant (Pogonomyrmex badius) is an attractant at low concentrations,

whereas at high concentrations it acts as an excitant, releasing the

typical aggresive posture and running of alarm behavior. It is un-

likely that carpenter ants in my study elaborated a pheromone('s) upon

exposure to x-rays, which in the spring acted as an attractant, be-

cause movement toward the beam was exhibited by antennectomized

ants, and by ants that were exposed individually in free-choice cham-

bers. The latter observation also ruled out influence by any type of

social facilitation.

There must have been physiological differences between these

ants in the spring and the fall of the year. In addition to differences

in responsiveness to x-rays, ants tested in the spring tended to

exhibit higher levels of activity and to disperse throughout the space

in which they were confined, rather than settling in a group, as was

typical of ants tested in the fall.

There is some evidence from the literature that supports this assumed physiologic difference. In some species of ants, workers undergo "certain physiological changes late in the season" according to Wilson (1963). Seasonal variation in locomotor and metabolic activity has been reported among workers of some species of Myrmica (Weir, 1958).

Many ants in temperate climates hibernate during inclement weather, including some species of Campanotus (Schneirla, 1951). With the onset of adverse weather conditions late in the fall, carpenter ants at my collection area began to retreat into the recesses of logs, and were not seen again in large numbers until March or April. In the summer of 1970 I established a laboratory colony of C. herculeanus, to provide subjects for research during the remainder of the study. Ants in this colony began to disappear in November and December, even though maintained under constant conditions of temperature and light. I assumed that the colony had been depleted, but, in February and March, between 50 and 100 ants emerged gradually from the log within the terrarium, leading me to suspect that worker ants had gone into true diapause during the winter months. Additional review of the literature revealed another study in which workers of northern carpenter ants were found to undergo diapause, and other rhythmical phenomena, independent of ambient temperature

(Hoelldobler, 1961).

If worker ants of C. herculeanus (and C. laevigatus) exhibit an

annual period of spontaneous dormancy, they are apparently unique

among all species of ants for which this question has been investigated

(Wilson, 1963). The physiologic changes preceding and following dia-

pause, although not well understood, might be responsible for the dif-

ference in behavioral reactions to ionizing radiation at different times

of the year.

The lethality study was undertaken mainly to allay suspicions

that the prompt and long-term responses discussed above might in-

volve radiation damage. In continuous exposures, no immediate be-

havioral evidence of injury was observed until subjects had been ex-

posed to 80-99 kR. (The group receiving 50 kR did develop a transient

case of leg twitches after exposure, which lasted for about three days

[Table 6]). By comparison, during the study of prompt responses,

the maximum exposure received by most groups was 2. 4 kR (80 R/sec

for 30 sec). A few of the studies within free-choice chambers re-

sulted in delivery of up to 60 kR to the unshielded compartment (Table

5), but even when ants in these groups exhibited attraction to the beam

in the spring it is doubtful that any individual was exposed to 50 kR,

since leg tremors never appeared in any of these ants. These and

other reasons, discussed above, provide evidence that damage was

not involved in the prompt behavioral and electrophysiological

reactions, nor in the behavioral responses of escape, conditioned spatial avoidance, and attraction.

The lethal quantities of radiation for carpenter ants are of the same magnitude as those reported for other hymenopterans. According to Ducoff, et al. (1971), doses above 50 kR have usually been necessary to reduce the longevity or to adversely affect physiological parameters of adult diptera and hymenoptera. A study of lethality in the pharoah ant, Monomorium pharoani, showed that 140 kR gamma radiation killed half, and 225 kR all, of the workers within 24 hours (Cole, et al. 1959).

These amounts of radiation are orders of magnitude higher than amounts necessary to cause the death of various mammals in comparable periods of time (Casarett, 1968). For example, whereas the calculated LD 50/30 $_{days}$ for carpenter ants was 100 kR, this parameter is less than 1 kR for all mammalian species that have been studied; as little as 200 R causes death within 30 days of half of a population of guinea pigs.

One major reason for this large difference in radiosensitivity is presumed to be that adult hymenopterans lack cell renewal systems, such as the hematopoetic system and the mitotically-active lining of the gut; in mammals these two systems are extremely vulnerable to damage by ionizing radiation. The body of adult hymenopterans consists chiefly of nerve, muscle, and fat (encased in a nonliving

cuticle); these tissues are notably radioresistant.

Doses in excess of 10 kR lead to death in mammals within a few hours to a few days (Casarett, 1968). The terminal behavioral symptoms, including loss of coordination, ataxia, and intermittent stupor, are roughly similar to the behavioral syndromes described for ants exposed to massive doses of 100 kR or more. In both of these instances, all animals die, and the behavioral manifestations of injury suggest that damage to the central nervous system is the primary cause of death.

SUMMARY AND CONCLUSIONS

1. Carpenter ants gave immediate, transient behavioral reactions
    to x-rays at exposure rates ranging from 0.05 R/sec to 80
    R/sec. Within this range, latency of a response was inversely,
    whereas strength, duration, and percent subjects responding
    was directly proportional to the exposure rate.

2. Comparisons of behavioral reactions of normal subjects with
    subjects whose eyes or antenna had been removed or shielded
    revealed that sensory receptors, probably olfactory, on the
    antennal flagella were responsible for detection and led to the
    onset of prompt responses. The visual system was more im-
    portantly involved in the transient cessation of activity (the
    "off" response) that occurred at the termination of exposure.

3. Bioelectric responses recorded from isolated eyes (ERGs) and
    from isolated antenna (EAGs) largely corroborated evidence
    derived from behavioral studies that these two sensory sys-
    tems are sensitive detectors of ionizing radiation. The
    strength of the induced bioelectric potentials varied directly
    with stimulus strength. Approximate threshold exposures or
    doses, delivered rapidly in fractions of a second, were: ERG
    response to beta radiation, 3.2 mrads; to x-rays, less than
    30 mR; EAG response to beta radiation, approximately 200

mrads.

4. Whereas x-ray and visible light stimuli elicited nearly identical
    ERGs suggesting similar mechanisms of action on the photo-
    pigment in the omatidia, the ERG responses to beta radiation
    were qualitatively and quantitatively different from the above.
    The reasons for this disparity are not apparent. Similarly,
    there were unexplainable differences between beta radiation-
    induced EAGs and odor-induced EAGs.

5. Ants escaped from and avoided prolonged exposures to 20 - 80
    R/sec x-rays when tested in the fall of the year. Using ioniz-
    ing radiation as the unconditioned, aversive stimulus it was
    possible to establish a strong conditioned avoidance response
    toward the place of exposure (rough-floored compartment of
    the free-choice chamber). This compartment was initially pre-
    ferred by the experimental subjects.

6. Exposures under identical conditions in the spring of the year
    resulted in the movement of ants into, rather than away from,
    the radiation field. Attempts to condition a positive or negative
    response toward the place of exposure failed. Seasonal differ-
    ences in the latency and strength of prompt reactions appeared
    as well.

7. Seasonal differences in responsiveness were shown to be unre-
    lated to potential pheromone, and phototrophic, effects. The

evidence from the literature and the laboratory strongly suggests that ants are physiologically different at these different times of the year, but the basis for such differences is not understood.

8. The motivational effects of ionizing radiation are apparently independent of mechanisms responsible for prompt detection, since ants exhibited attraction to the beam during prolonged exposures in the spring even in the absence of antennal and visual receptors. Operated subjects were not studied in the fall.

9. Neither prompt responses or motivational effects were due to radiation-induced damage. These behaviors were reversible, and were exhibited with exposures ranging from a few mR (bioelectric responses) to a few kR (motivational effects). By contrast, behavioral evidence of injury did not appear during continuous exposure until at least 82 kR had been delivered.

10. In lethality studies, the time of death and the severity of behavioral symptoms of damage were dependent on total exposure. The LD $50/30_{days}$ was 101 kR, and the LD $50/60_{days}$ was 66 kR. These were not totally useful expressions of damage since all ants exposed to 50 kR or more eventually died prematurely; there was no recovery, as is typically found in mammals in

the midlethal dose range. Behavioral syndromes developing

with exposures to massive amounts of radiation indicated that

injury and death resulted primarily from damage to the ner-

vous system.

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