The light-adapted crayfish, Pacifastacus trowbridgii (Stimpson, 1957), displayed a behavioral response to exposure to 300 kV x-rays at exposure rates of 10 to 30 R/s. Within this range, the proportion of subjects that responded increased with an increase in exposure rate. The response latency was inversely proportional to the exposure rate. Ophthalmectomized animals exhibited a similar response with a significantly shorter latency than the intact animals at the same exposure rate (30 R/s). Partial body exposure of ophthalmectomized animals also elicited a behavioral response and indicated that a radiation-sensitive receptor was located in the abdomen.

X-ray exposure of the dark-adapted compound eye evoked an electroretinogram (ERG) that was similar to the light evoked ERG. The x-ray evoked ERG amplitude was found to be dependent on total exposure for stimulus durations of 300 ms or less. With stimulus durations greater than 300 ms, the ERG amplitude increased in relation to the logarithm of the exposure rate. Similar responses with
light, indicated that the mechanism of interaction may be the same for x-rays. The time course for maximal dark-adaptation, after a 500 ms exposure to 3.85 ft-c of light, was comparable for both x-ray and light exposure (9 min). Differences observed in ERG amplitude between the light and x-ray evoked responses during the initial recovery period can be attributed to absorption of light by migrating accessory pigments or by differential interaction of light with photosensitive pigments in the eye.

X-ray exposure (10 to 35 R/s) of the medial branch of the antennule and the cheliped of the first walking leg did not yield any significant chemoreceptor responses as judged by electrophysiological tests. The presence of chemoreceptors was indicated by responses elicited after administration of glutamic acid, glycine, and fish extract.

X-irradiation of the dark-adapted sixth abdominal ganglion in both isolated and in vivo preparations elicited similar increases in neural impulse frequency. Significant increases occurred after ten seconds of exposure with an exposure rate of 25 R/s. The response latency decreased to five seconds with 30 and 35 R/s. It was observed that the spike potentials evoked with x-rays were similar to those evoked by light (75-100 microvolts). There was, however, a supplemental increase in spike potentials of lower amplitude (40-50 microvolts) during x-irradiation that was not observed with light. This indicated that neural elements other than the photosensitive neurons of the ganglion were activated. It appears
likely that the behavioral response in crayfish, subjected to abdomen-only exposure may be instigated by x-ray excitation of the sixth ganglion.
BEHAVIORAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR THE MECHANISMS INVOLVED IN THE DETECTION OF IONIZING RADIATIONS BY THE CRAYFISH PACIFASTACUS TROWBRIDGII

by

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The love, patience, and motivation my wife, Jane, offered are primarily responsible for my efforts in this endeavor. To my children, Nicole and Nathan, and our future, I dedicate this thesis.
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INTRODUCTION

Behavioral Responses Induced By Exposure to Ionizing Radiations

The first observations on the detection of ionizing radiations by organisms were made by Axenfeld (1897a & b). He observed that certain insects and crustaceans, placed in a partially-shielded, light-attenuated box, would migrate to the unshielded portion during x-ray exposure in a free choice situation (shielded versus unshielded). Subsequent studies have revealed that many organisms react to the process of irradiation by increased appendage activity or by responses that are characteristic of various forms of neural stimulation. For example, snails (Helix pomatia, Arion empiricorum, and Helicella candidans) were observed to respond to 50 kV x-rays at exposure rates of 1.5 to 5 R/s by retraction of their tentacles (Hug, 1958). Several annelids were found to respond by increased peristaltic contractions (leech, earthworm, and Tubifex) at relatively low exposure rates, and eventually moved out of the field of irradiation with prolonged exposure and increased exposure rates (Bergeder and Boning, 1965; Hug, 1958). Bergeder and Boning (1965) did not detect much evidence for a relationship between the sensitivity and the extent of phylogenetic development but their studies were not very systematic. The observed animals were from the phyla containing annelids, molluscs, arthropods, and included
only one vertebrate. Behavioral responses occurred to 50 kV x-rays with exposure rates which ranged from 5 to 1350 R/s and total exposures of 60 to 1000 R across the variety of forms tested. The annelids and the single vertebrate tested (grass frog) seemed to be least sensitive. The arthropods showed the greatest variability in responsiveness as a group, and the insects in general showed the greatest sensitivity.

While Hug and Bergeder and Boning did considerable work on the detection phenomenon, in most cases they failed to describe their methods, the number of animals tested, the proportion that displayed a positive response, or any statistical analyses of the data. This lack of technical and quantitative information makes it difficult to draw conclusions and to compare the sensitivity of the organisms phylogenically.

In the sea anemone, Anthopleura xanthogrammica, it has been determined that some of the responses with x-rays (Kimeldorf and Fortner, 1971) are similar to responses with monocromatic radiations in the UV and visible range of 240 to 650 nm (Clark and Kimeldorf, 1970, 1971). The responses observed were tentacle and body retraction that lead to complete closure of the oral-disc region. The reaction time with x-rays (300 kV) was found to be inversely proportional to the exposure rate and ranged from 102 seconds at 20 R/s to 266 seconds at one R/s. Dedrick and Kimeldorf (1974) studied tube foot retraction and spine flexion in an echinoderm (Strongylocentrotus purpuratus) as responses to
x-ray exposure rates of one to fifteen R/s. Reactions times varied inversely with exposure rate in the sea urchin. It was also observed that the response occurred in shielded parts of the animal when only partial body exposure was employed. Analysis of the reaction times and nerve transection studies implicated the subdermal nerve net as well as the radial nerves in reactions in shielded areas. The effective exposure for 50 percent of the animals to respond with tube foot retraction was 3.6 R at 1 R/s. Martinsen and Kimeldorf (1972a & b) studied the response of carpenter ants to ionizing radiations. The ant was found to be very sensitive and exhibited behavioral responses (antennal movement) at exposure rates as low as 0.05 R/s. The ants would also avoid prolonged exposure to 20 to 80 R/s if allowed to migrate into a shielded area.

Vertebrates can also detect ionizing radiations. Prompt, immediate behavioral responses have been observed in the laboratory rat. Hunt and Kimeldorf (1962, 1963, 1964) observed that exposure to x-rays of low intensity, 0.05 R/s, immediately aroused the sleeping rat. It was found that the immediate response was exposure rate dependent, and was accompanied by transient changes in Electrocuccephalogram (EEG), cardiovascular, and respiratory states. This phenomenon of detection has been observed in a variety of species, including man.
Sensory Pathways Involved in Immediate Detection

Photosensory Mechanisms

There is considerable evidence, both behavioral and electrophysiological, that shows the involvement of photo-receptors in the immediate detection of ionizing radiations. The earliest evidence that implicated the visual route came from Axenfeld's experiments with insects and crustaceans (1897a):

If insects (Coleoptera, Diptera, Hymenoptera) or crustaceans (Porcellius) are taken in a box, half of which is made of wood, half 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; therefore one may be lead to assume every possibility of a sense of sight, for blinded animals do not show this capacity.

Stimulation of photoreceptors has been postulated as the primary mechanism in the sea anemone (Kimeldorf and Fortner, 1971) and the sea urchin (Dedrick and Kimeldorf, 1974), since similar behavioral responses were obtained with visible radiations. The water flea, Daphnia magna, reacted to x-rays in a similar manner as with light, and swam downward when exposed (Baylor and Smith, 1958). The dark-adapted fiddler crab ceased motor activity when irradiated at 2 R/s and this "off" response did not occur when the animals were blinded (Terwilliger and Levy, 1964).

Other evidence for the visual route of detection comes from electrophysiological studies. An electroretinogram (ERG) has been elicited for the dark-adapted eye by ionizing radiation of both
vertebrate and invertebrate organisms. For the isolated frog retina, Lipetz (1955) calculated the absorbed energy to be 0.1-1.7 x 10^-3 ergs for light and 4 x 10^-3 ergs for x-rays. Electrophysiological studies on invertebrates have shown that the compound eye of arthropods is also sensitive to ionizing radiations. Electroretinograms have been produced in the cockroach (Baldwin and Sutherland, 1965; Baldwin et al., 1963), the purple shore crab (Jordan and Kimeldorf, 1971), Noctuid moths (Smith and Kimeldorf, 1964), the carpenter ant (Martinsen and Kimeldorf, 1972b), and the red ghost shrimp (Kernek and Kimeldorf, 1975). The ERGS were obtained with x-rays and beta-radiation and appeared to be similar to stimulation with light. In general, dark-adapted, scotopic receptors are more sensitive than photopic receptors. Table 1 is a summary of bioelectric responses obtained from invertebrates, including ERGs.

Chemoreceptor Mechanisms

The chemosensory systems are also highly sensitive amplifying systems. Olfactory receptors can detect traces of chemicals in the environment in concentrations of less than one part per million. The sensitivity of the olfactory system to ionizing radiations was first discovered in the laboratory rat. Experiments by Kimeldorf and Hunt (1965) determined that ophthalmectomized rats could be aroused from sleep with a brief x-ray exposure and indicated that the visual mechanism was not essential to detection.
Partial body exposure of the head in blinded animals elicited a greater incidence of arousal and a more pronounced reaction than other body regions (Hunt and Kimeldorf, 1963). Later experiments by Cooper and Kimeldorf (1966) and Cooper (1968) found that the olfactory bulb was essential for radiation detection in blinded animals. Spike potentials of neurons in the olfactory bulb were increased during irradiation, and desynchronization of the EEG occurred. When the olfactory bulb was ablated, there was no response or desynchronization of the EEG pattern. Subsequent studies have demonstrated that the olfactory epithelial receptors act to mediate detection. If the receptors were destroyed with ethyl alcohol perfused through the nasal cavities, the olfactory bulb did not respond to irradiation.

Detection of ionizing radiations was also found to be mediated by olfactory receptors in the carpenter ant. These animals would respond to ionizing radiation even if blinded or if they were light-adapted so as to prevent visual detection (Martinsen and Kimeldorf, 1971, 1972a & b). At least seven distal segments of the antennae, which make up part of the olfactory sensilla, must be intact to insure a behavioral response. Electroantennographic (EAG) responses were obtained with 150 to 380 mrads/s of beta-radiation from a Sr-Y-90 source (Table 1). Kernek and Kimeldorf (1975) observed that EAGs and bioelectric potentials from the swimmeret could be elicited upon irradiation with x- and beta-radiation, in the red ghost shrimp. Previous responses cited for animals
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that were not dark-adapted (Bergeder and Boning, 1965; Hug, 1958, 1960; Hug and Schliep, 1961) suggest that a non-visual route may mediate detection in several circumstances.

Other Potential Receptor Systems

Other receptor systems have not been studied extensively in terms of the ability to be directly excited by ionizing radiation. These systems include mechano-receptors, free nerve endings, internal chemoreceptors, and direct stimulation of the central nervous system. Kimeldorf and Hunt (1965) cite a few cases in which these other types of receptors exhibited immediate responses to irradiation. A prompt blink reflex was elicited upon exposure to the cornea of an unanesthetized rabbit to a two ms burst of alpha particles with a threshold of two krad (Tobias et al., 1962). Exposure was limited to the level of free nerve endings in the cornea; however, the massive dose required for stimulation, indicated that free nerve endings are relatively insensitive.

Mechanoreceptors are highly insensitive to ionizing radiations, however beta-irradiation of the Pacinian corpuscle of the cat, altered its sensitivity to mechanical stimulation (Talbot et al., 1969; Talbot, 1970).

It is possible that the neural synapse may act as a receptor, since in many ways it is a chemical transducer. The central nervous system (CNS) has a great number of synaptic connections, and it might be speculated that direct neural excitation occurs. This
possibility is difficult to explore directly. Although changes in EEG have been recorded as a result of exposure (Kimeldorf and Hunt, 1965; Garcia et al., 1963), many of these changes are thought to be due to peripheral stimulation of receptors. Hug and Schliep (1963) studying radiation-induced muscle contraction in the leech, found that isolated muscle that was completely freed from the nerve cord, required higher doses and dose rates to produce the same effect as in the intact nerve-muscle preparation. This possibly suggests involvement of neural elements in the nerve cord or the myoneural junction.

Statement of the Present Problem

There is little information on the ability of freshwater crustaceans to detect ionizing radiations and the routes of detection. Studies cited previously (Terwilliger and Levy, 1964; Hug, 1960; Bergeder and Boning, 1965; Axenfeld, 1897a & b) have shown that at least some crustaceans detect ionizing radiation and the investigators have assumed that visual receptors are the main route of detection. However, the possibility of non-visual detection in crustaceans has not been explored sufficiently to determine the extent of generalization possible. Chemoreception has been observed in ants (Martinsen and Kimeldorf, 1972b). Also, a recent study suggests the possibility of non-visual routes in the red ghost shrimp (Kernek and Kimeldorf, 1975).

There appears little doubt that many organisms can detect ionizing radiations and that it is a widely distributed phenomenon.
The emphasis is presently placed on investigation of the sensory routes or neural mechanisms which mediate detection. In the present study the ability of the crayfish to detect ionizing radiation was assessed. In this highly evolved form, non-visual routes of detection were explored and investigations were made of chemoreceptors and the photoreceptor neurons in the sixth abdominal ganglion (Prosser, 1933) as well as the visual routes.

**Objectives of This Study**

The specific objectives of this study are: (1) to determine by behavioral criteria, if the crayfish can detect ionizing radiation; (2) to determine the radiation exposure parameters that affect detection, if the animal is capable of detection; (3) to use sense organ isolation and/or localized exposure as a means of defining critical routes; (4) to investigate by means of electrophysiological methods the principle sensory systems for vision, olfaction, or chemoreception as routes of x-ray detection; (5) to determine if the sixth abdominal ganglion may be excited by ionizing radiation, and (6) to identify and characterize any response of these receptor systems as a function of the physical parameters of exposure.

**Justification for Using the Crayfish**

The crayfish was chosen for this study because this animal possesses a variety of well defined receptors on its surface. Chemoreceptors have been studied by various workers in related crustaceans. Hodgson (1958, 1968) found that amino-acid (glycine
and glutamic acid) receptors are found on the medial branch of the antennule and the chelipeds of the first two pair of walking legs. Recently it has been determined that chemoreception may play a greater role in crustaceans than was believed previously. McLeese (1970) discovered that olfactory attraction occurs between lobsters. Kettredge, as quoted by Sutterlin (1974), found that petroleum pollutants completely inhibited feeding and pheromone-mediated sex responses in the crab, *Pachygrapsus crassipes*. Olfactory receptors have been shown to mediate the detection of ionizing radiations in the carpenter ant and red ghost shrimp and suggests the possibility that chemo-receptors may serve as a major route of detection, at least in invertebrates. It would appear that olfactory receptor and taste receptor responses could be studied in the crayfish as possible routes of detection of ionizing radiations in addition to photo-reception.

The discovery of the photosensitivity of the sixth abdominal ganglion in the crayfish by Prosser (1933), suggests that this could be another possible route of detection in this crustacean.

In addition, it is possible to develop consistent and reliable behavioral criteria of a prompt response to ionizing radiation in the crayfish.
MATERIALS AND METHODS

Classification of *Pacifastacus trowbridgii*

The classification of the crayfish of western North America has undergone several revisions and there has been much confusion as to their proper status. They were originally classified under the genus *Astacus*, the same genus as the European crayfish. In 1950, Bott revised the sub-family Astacinae and identified the European crayfish in the genera *Astacus* and *Austropotamobius* and a new genus *Pacifastacus* for the western North American group (Miller, 1960). The sub-family Astacinae is one of two in the family Astacidae in the northern hemisphere, and is characterized by 18 lateral gills and non-specialized copulatory organs. There are four genera in this sub-family: *Astacus* and *Austropotamobius* are European; *Pacifastacus*, western North American; *Cambaroides*, Siberian and Japanese. The other sub-family, Cambarinae, has six genera in eastern North America and have 17 lateral gills and highly specialized copulatory organs. According to Mason (1963), Bott based his revision on the morphology of the first and second gonopods, spine counts on the merus segment of the third maxilliped, and the absence of spines or ridges on the epistome.

The crayfish used in this study was originally described by Stimpson in 1857 as *Astacus trowbridgii*, the genus that was later revised to *Pacifastacus*. The animal in this study will be referred to as *Pacifastacus trowbridgii* (Stimpson).
Life History

Observations on the life history of *Pacifastacus trowbridgii* (Stimpson) have been made by Miller (1960), Mason (1963), and Coykendall (1972). These animals are perennial Fall breeders; the exact timing of breeding is variable and depends on the particular location, population, and climate associated with the particular stream or body of water. Mating occurs from September to late October. The males deposit long, string-like spermatophores on the venter of the female, near the gonopores. Deposition of spermatophores is followed shortly by extrusion and fertilization of the eggs.

The incubation period in *Pacifastacus* sp. is long (Coykendall, 1972) and lasts from five to seven months during winter. The eggs are carried on the pleopods of the female and hatch in late Spring and early Summer, depending on the temperature. Growth is slow in northwest waters, the larger males mature in 17 months and females in four years (Coykendall, 1972). After hatching, the young stay attached to the female, and live off the stored yolk reserves. The young leave the mother soon after the first moult and forage on their own. The young crayfish (subyearlings) moult eight to fifteen times during the first year of life (Miller, 1960; Mason, 1963). Adults over 70 mm in length moult, on an average, once a year.

Migrations of *Pacifastacus trowbridgii* in a coastal stream have been found to occur in late Fall and early Spring (Miller,
1960). Downstream migration occurs from April to June and involves primarily females, whereas upstream migration occurs from September through November and involves fewer animals but approximately equal proportions of males and females. During the winter months the activity of the crayfish decreases and may cease as a result of lower water temperatures. Miller (1960) found that catch per unit effort decreased to nearly zero at 3.2° C.

Collection of the Experimental Animal

Crayfish were collected from local streams and rivers in the surrounding Corvallis area. The primary collection sites were the south fork of the Alsea River and Rock Creek, which is a drainage stream of the Mary's Peak water shed. The animals were collected from early Spring to late Fall, the prime collection period being from July to September. Collection was done in the late afternoon when the animals were more active. Various methods of collection were employed, and included baited traps; however the most successful method was to capture them with the use of a long handled dip-net and a long stick used to prod the animals into the net. In areas where crayfish were not readily observed, pieces of fish or liver bait were placed along the bottom of the stream; one could then capture the animals which were attracted to the bait.

At the collection site, the animals were placed in a covered styrofoam ice-chest, filled with stream water. The water in the container was periodically changed to keep the animals from suffer-
ing from oxygen depletion. The animals were thoroughly rinsed and fresh water added just prior to transport to the laboratory.

**Maintenance in the Laboratory**

The crayfish were kept in two 150 gallon Instant Ocean aquaria, filled with dechlorinated tap water. The temperature of the water was thermostatically regulated and maintained between 14° and 16° C. This temperature was approximately the same as that which occurred in the natural environment in mid-Summer. A quarter of the water in the tanks was periodically removed and replaced with fresh dechlorinated water. This was done primarily to maintain the water conditions as constant as possible and to compensate for any loss due to evaporation.

The crayfish were fed a variety of foods twice weekly. Since they are omnivorus, the animals were fed pieces of frozen flounder, shrimp, beef liver, and supplemented with chopped carrots and dried cat food.

The animals were kept on a twelve hour on, twelve hour off, light-dark cycle. An automatic timer and switch was used to control two banks of fluorescent lamps (Westinghouse F40/Grow-Lights) placed directly over the aquaria.

Approximately 40 to 50 crayfish, six to 15 cm from telson to rostrum, could be kept in each tank throughout the year. Periodically a number of animals would moult and these animals were then placed in individual containers, which allowed them protection and time for the exoskeleton to harden.
Studies of Radiation-Induced Behavioral Responses

The initial studies were aimed (1) to determine if the light-adapted crayfish could respond to an x-ray exposure, (2) to characterize the response, and (3) to determine reaction time or latency of response from the onset of exposure. Various exposure procedures and dimensions of radiation exposure chambers were tested. It was found that if the animals were placed in a chamber filled with water, they would move about, explore the chamber, and make it difficult to identify a specific behavioral response as a result of x-ray exposure. The use of various systems to restrict movement was also unsatisfactory, since the animals would "freeze" in posture and a response could not be elicited unless the chamber was moved or tapped. It was observed, however, that a non-restrained crayfish in a water-filled chamber, that was motionless, would promptly move after onset of x-ray exposure. Experiments were then done on animals placed in the chamber without water. A piece of wet paper towel was placed on the bottom of the chamber to maintain the atmosphere saturated with moisture. Under these circumstances, the animals would become quiescent after the initial 5-10 min of exploration. This arrangement proved to be the best method for observation of the animals.

The crayfish used in the experiments ranged in size from eight to 13 cm in length, measured from the tip of the rostrum to tip of telson. They were placed in a covered clear acrylic plastic chamber with dimensions of 8.3 x 8.3 x 15.3 cm with six mm thick
walls. The chamber was placed over three inches of foam rubber pads that rested on a wooden table top.

Response Criteria. The crayfish generally settled down and ceased gross motor activity within five to ten min after placement in the chamber. A resting position (Figure 1) was assumed and was characterized by the animal lying flat on the bottom surface of the chamber with the walking legs and chelae extended. The animals could maintain this position, for up to five minutes.

The criteria developed and used to assess the occurrence of a behavioral response to ionizing radiations consisted of a rapid sequence of movements. The initial alerting events, which signalled behavioral arousal, involved an elevation or flexion of antennae, the chelae were raised, and the entire body was lifted on all walking legs. Subsequent to this alerting response was an abrupt increase in general motor activity in the chamber. This was the typical behavioral pattern whenever an animal initiated motor activity from the resting position. This behavioral sequence was used as the criterion for a positive response in irradiated and in shielded (sham-irradiated) control animals.

Observation and Recording Methods. The crayfish were allowed to explore the chamber for five to ten minutes, until they settled down. If they were quiet for the criteria time of one minute, recording and exposure would begin. The animals' responses during irradiation were observed with the use of a closed circuit TV camera (Shibaden HV-15) and video monitor. A video tape recorder
Figure 1. The crayfish, *Pacifastacus trowbridgii*, in the exposure chamber, assuming a typical resting position.
(Apeco) was used to record the animals behavior from one minute prior to exposure and continued for one minute post exposure. Subsequent observation of the tape recording could be done to extract the necessary information for quantitative analysis. The proportion of respondents for the irradiated and sham-irradiated control groups was determined for the duration of the exposure and for the post-exposure period. The time it took the animals to begin motor activity (latency) from the onset of exposure was recorded.

X-ray Exposure and Dosimetry. A General Electric Maxitron 300 kVp x-ray therapy unit was used for all exposures. For these studies, the machine was operated at 300 kV, and 20 mA, with a beam filtration equivalent to a half-value layer of 0.25 mm of aluminum. Exposure rate could be varied by changing the target to subject distance.

All x-ray exposure rates were determined with the use of a Victoreen thimble ion chamber and R-meter. Readings were taken inside of the exposure chamber in air. The x-ray target-subject distance was adjusted until the proper exposure rate was attained.

Surgical and Exposure Procedures

Ophthalmectomy. The crayfish were anesthetized in an ice-bath for 15 minutes prior to bilateral removal of the eyes. The entire eye cup and stalk was severed at its base with a fine pointed surgical blade. Each animal was housed in a plastic container
and returned to the 150 gallon tank. The crayfish were allowed four days to recover from the operation prior to test under the experimental conditions.

**Partial Body Exposure.** Ophthalmectomized animals were placed in the chamber and allowed to explore until the animal came to rest with the abdomen in the extended position. A lead shield on a wooden rack was elevated above the exposure chamber so as not to make physical contact. The lead shield was then positioned over the cephalothorax and covered all the walking legs. In this manner, the shield was positioned without any vibration or noise that might disturb the animal.

**Procedures For Determination of Radiation-Induced Avoidance Behavior**

The experimental arrangement for determination of an avoidance response to x-rays consisted of a chamber partitioned into two sections [in half] by a lead shield (Figure 2). The chamber dimensions were 38 x 15 x 10 cm. The lead partition extended into the chamber and allowed clearance of 2.5 cm for the crayfish to pass beneath. The water depth was 2.5 cm and just touched the lead shield. The portion of the lead partition that extended into the chamber was given three coats of non-toxic, clear lacquer to prevent possible lead contamination. One half of the chamber was exposed to the room light that consisted of two 300 watt incandescent lamps and the other half was shielded from light by placement of a black paper over it. White paper was placed under the
Figure 2. Diagramatic representation of the experimental set-up for determination of x-ray avoidance. Not drawn to scale.
illuminated half and a sheet of paper painted flat black was placed under the darkened half. The light intensity in the illuminated side was 35 ft-c and in the other section it was two ft-c, as measured with a Weston model 703-67 meter. This experimental arrangement allowed the animal a free choice to establish a residence with respect to light and dim-light. The animal could then be tested with x-rays to determine if avoidance against normal preference for low illumination could be established.

X-ray measurements and dosimetry were made with a Victoreen thimble ion chamber inserted into a mass of lucite plastic of thickness equivalent to the water in the exposure chamber. The x-ray tube head was centered over the darkened portion of the chamber and yielded an exposure rate of 30 R/s. The exposure rate in the illuminated side ranged from 0.36 R/s at four cm from the lead partition to 0.05 R/s at four cm from the far end wall. As a control for Sham-exposure, a horizontal lead shield was placed over the light attenuated side.

The crayfish were tested individually. They were introduced at alternate ends with respect to the center partition. The animals were allowed to explore the chamber for five minutes prior to observation and exposure. When the animals had been in the light-attenuated half for at least one minute the test was initiated. Each animal was observed for five minutes under control conditions, followed by five minutes exposure in the light-attenuated portion, then a five minute post exposure period. The activity of the animals was observed with the use of the television equipment.
described earlier and recordings of the test were made on videotape. The latency of escape was determined from onset of x-ray exposure to the initial movement to the illuminated side. The total time spent in the dim-side was recorded for each five minute period.

Electrophysiological Methods

The Electroretinogram (ERG)

Surgical Methods. The eye was removed by the methods described in the section on ophthalmectomized crayfish and studied as an isolated preparation. The corneal surface was pierced with a fine insect pin and the eye was placed in a light-tight container, filled with chilled Van Harreveld solution (Van Harreveld, 1936) for about 15 minutes prior to placement of the eye on electrodes.

Recording Methods. Two silver-silver chloride suction-type, glass electrodes, filled with Van Harreveld solution were used to record the electroretinogram (ERG). The base of the eye cup was mounted on a reference electrode with a tip diameter approximately the same size as the base of the eye. Slight suction was applied, which served to hold the eye securely and make electrical contact with the tissues. The recording electrode, with a fine beveled tip, was carefully inserted into the puncture on the cornea, approximately two mm, with the use of a micromanipulator. The electrical activity of the eye was recorded differentially with a Grass model 7P5A-AC preamplifier in line with a model 7D-AC
oscillograph driver amplifier and recorded with a Grass model 8P-A ink-writing four-channel oscillograph. The eye could last up to three hours in this manner without too much loss of ERG amplitude. Periodically, Van Harreveld solution was used to rinse the eye to reduce excessive drying. All studies were done on fresh preparations in this manner. Electrotretinograms recorded from surgically isolated eyes have been shown to be identical to responses of the intact eye (Hartline, 1928). All eye preparations were dark adapted for 30 minutes before the test with either light or x-rays. It was found from response amplitude measurements that after 30 minutes, the eye was fully dark-adapted.

**Exposure Methods.** All x-ray exposures were done with the Maxitron unit operated as described for the behavioral studies. The exposure rate was changed by changing the tube current (Table 2). All exposures were measured in the air with a Victoreen thimble.

<table>
<thead>
<tr>
<th>Exposure Rate (R/s)</th>
<th>Tube Current (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>2.5</td>
</tr>
<tr>
<td>1.7</td>
<td>5.0</td>
</tr>
<tr>
<td>3.3</td>
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<td>15.0</td>
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<tr>
<td>7.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>
ion chamber and R-meter. The sensitive volume of the ion chamber was placed in the same geometrical position as the eye in the exposure chamber.

The preparations were exposed in a light-tight, lead-lined box that served as a Faraday's cage. The inside of the box was lined with aluminum foil and all surfaces were painted flat black to absorb any fluorescence that might occur during x-irradiation. The top of the box had a 2.5 cm diameter port through which light and x-rays passed. The stimulus duration was controlled with a solenoid-driven, focal plane lead shutter. Due to the inertia of the lead shutter, pulses less than 70 ms duration or greater than four pulses per second could not be obtained reliably. A photoresistor cell placed beneath the preparation was used to monitor the stimulus (Figure 3).

The light source was a General Electric number 62 incandescent lamp, powered by a six volt dry cell battery. The light intensity was controlled by adjusting the filament current with a potentiometer. Figure 4 shows the effect of different potentiometer settings (increased resistance) on the light intensity at 24 cm from the surface of the light source. Light intensity was measured in ft-c with a Weston model 703-67 meter. The light output of the lamp decreased exponentially with increased potentiometer setting. Most light intensities used on the eye were below the levels which could be measured with the ft-c meter and were extrapolated from the graph.
Figure 3. Diagramatic representation of the experimental set-up for exposure of the isolated crayfish eye. Not drawn to scale.
Figure 4. Light intensity of a G.E. number 62 incandescent lamp powered by a six volt DC power source. Intensities are measured at the distances indicated, from the tip of the optic fibers and the lamp surface to the surface of the light meter (Weston model 707-63). The potentiometer settings are in arbitrary units and represent increasing resistance and decreasing filament current.
Without optic fibers, 24 cm distance

Light passing through optic fibers, 2 cm distance

Light intensity in foot candles vs. potentiometer setting.
Quantitation of the ERG. The amplitude of the ERG "on" wave was used to study the effects of different stimulus parameters. The data obtained on the ERG "on" wave was normalized by expressing the amplitude as a fraction of the maximum amplitude obtained under standard conditions of stimulation. The ERG amplitude when exposed to a 500 ms pulse of x-rays at 7.0 R/s, yielded a maximal response and served as a standard. In this way, the relative response of the ERG could be compared when the eye was subjected to different stimulus parameters. The ERG response of the eye has been found to decrease slowly as a function of the time after removal from the purple shore crab (Jordan, 1971). The drop in biological activity was probably due to trophic factors, anoxia, hormonal changes, and damage produced during removal and placement of electrodes. For this reason, the response of the crayfish eye was periodically checked with the standard stimulus before and after a series of exposures.

Dark-Adaptation Methods. To determine the time course of dark-adaptation for both light and x-rays, the light source was adjusted, in intensity, to yield an ERG equal in amplitude to that obtained with a standard 500 ms x-ray exposure at 7.0 R/s. With ERG amplitudes for light and x-rays equated, the eye was subjected to a 500 ms light flash at an intensity of 3.85 ft-c. The eye was then tested at intervals with x-rays or light until an ERG amplitude was obtained which was equal to the ERG just prior to the light flash.
**Isolated Chemoreceptors**

**Antennule Preparation.** The medial branch of the antennule was removed from the crayfish with a fine scissors at its junction with the second distal, basal segment of the antennule. The flagellum was placed in a groove in a block of paraffin, so that the cut end extended into a small chamber at one end that was filled with Van Harreveld solution. Silicone grease (Dow Corning) was used as a dam to hold the solution in the chamber and to separate the cut end from the rest of the flagellum (Figure 5). The paraffin block was cut so that the longitudinal axis of the flagellum was inclined. This allowed stimulatory solutions to flow over and off the preparation.

A platinum clad (Grass E2) reference electrode was placed in the chamber filled with Van Harreveld solution. The recording electrode was a fine hooked, stainless steel wire, that was placed under the extended flagellum, with the aid of a micromanipulator. The stainless steel electrode was an insulated wire that was ground to a fine point on one side and bent to form a hook. The inside of the hook was the recording surface and the rest remained insulated.

**Walking Leg Preparation.** The animals were anesthetized in an ice bath for 15 minutes prior to removal of the first walking leg. The leg was excised with scissors at the point of autonomy with little blood loss. The appendage was placed in chilled Van Harreveld solution and the exoskeleton of the meropodite was
Figure 5. Diagramatic representation of the essential components for x-ray exposure of the medial flagellum of the antennule. Not drawn to scale.
split in half, longitudinally. The centrally located nerve was isolated with the aid of fine glass probes, under a dissecting microscope. After careful removal of all extraneous connective tissue and muscle, the large nerve bundle was split length wise into four to six smaller bundles with the aid of a fine pointed surgical blade and glass probes. Care was taken not to stretch the nerve excessively as this could damage the nerve fibers.

The leg was placed in a modified 30 cc plastic, tissue culture flask (Falcon Plastics), filled with Van Harreveld solution. The chela and part of the carpus were pushed through a rubber dam which covered an opening in the end of this chamber (Figure 6). This provided an excellent means of stimulation of the receptors located in the chela and carpus, that were outside of the chamber, without interference to the recording electrodes. The nerve bundles were teased apart in the solution of the chamber and each was picked up individually with a fine hooked, stainless-steel electrode until a bundle was obtained which contained a large proportion of afferent chemoreceptor fibers. The nerve bundle was frequently bathed with Van Harreveld solution to keep it moist. A platinum clad (Grass E2) reference electrode was placed in the bathing solution. The method described was similar to that used by Shelton and Laverack (1970), in their studies of chemoreception in the lobster.
Figure 6. Diagramatic representation of the essential components for x-ray exposure of the first walking leg. Not drawn to scale.
Recording Methods. Recording of nerve impulse activity (spike potentials) from the medial flagellum of the antennule and the walking leg was done differentially through a Grass model 7P5A-AC preamplifier in line with a model 7D-AC oscillograph driver amplifier. The signal from the amplifier was fed into and observed on a Tektronix 502A dual beam oscilloscope. A Grass model AM7 audio monitor was also used in conjunction with the oscilloscope to monitor the signal from the preparations. The electrical activity was recorded on a Tandberg series 100 four-channel magnetic tape recorder. The data could then be stored and played back later for study on a signal pulse height analyser (Ortec Model 406A) and scaler (Ortec model 430).

Chemical Stimulation. The preparations were subjected to chemical stimulation known to be effective in the crayfish (Hodgson, 1958). Solutions of 0.1 M glutamic acid and glycine were made up in pond water. A fish extract was made by macerating and filtering five grams of flounder in 100 ml of pond water. These solutions were all tested for effectiveness against pond water as a control blank. The water in which the crayfish resided, is referred to as pond water in this study. The solutions were dropped on the preparation with a fine tuberculin syringe. The preparation was rinsed thoroughly after each chemical administration. These solutions were used to verify the presence of chemoreceptors and to characterize their response for comparison with any response which might occur with x-rays.
X-ray Exposure. All exposures were done with the Maxitron x-ray unit operated as described for the behavioral studies.

The preparations were placed in a grounded, lead-lined box and positioned under a 2.5 cm port through which the x-ray beam passed. The x-ray beam was monitored with a photoresistor placed directly under the preparation. The entire antennule preparation, including the electrodes, were placed in the path of the x-ray beam. Only the chela and carpus of the leg were exposed to x-rays. The exposure duration was controlled by a timer located on the x-ray unit control panel. As a control, sham-exposures were done by shielding the preparation with lead and exposing it to 35 R/s. This was done to determine if the electrical noise from the x-ray unit would interfere with the biological signal.

The Abdominal Nerve Cord and Sixth Ganglion

Surgical Procedures. The electrical activity of the abdominal nerve cord and the sixth ganglion were recorded from isolated nerve preparations and from intact in vivo preparations. Crayfish, between 10 and 14 cm in length, were anesthetized in an ice bath for 15 minutes. With the aid of a dissecting microscope, the abdominal nerve cord was exposed and all sensory and motor connectives from the ganglia were cut and the cord between the first abdominal and last thoracic ganglion was cut. The nerve cord was removed and placed in chilled Van Harreveld solution at three to five degrees C. for 24 hours. This procedure greatly reduced the spontaneous
activity and possible injury potential effects. One could then observe the activity of the sixth abdominal ganglion without interference from activity of other ganglia.

For in vivo preparations, the crayfish were anesthetized and secured to a small wooden block with the abdomen extended and ventral side up. All of the legs were removed and the cephalothorax was wrapped in wet paper towels. The ventral abdominal exoskeleton and abdominal blood vessel were carefully removed to expose the nerve cord. The cord was desheathed and the surrounding connective tissue was removed, so that the cord and its connectives were left intact.

Recording Methods. Both preparations were placed in a grounded lead-lined box. The isolated nerve cord was placed in a wax chamber on the bottom of which was placed a strip of Kimwipe, saturated with Van Harreveld solution (Figure 7). Spike potentials were recorded differentially from two hooked, platinum clad (Grass E2) electrodes which lifted the nerve into the air with the aid of a micromanipulator. The preparation was periodically rinsed with fresh solution to reduce drying effects.

Activity from the intact preparation was recorded with a silver-silver chloride, fluid filled, glass suction electrode, in contact with the nerve cord. A reference electrode was embedded in the muscle tissue of the abdomen. To ensure that good contact was made with the nerve, the tip of the electrode was approximately the same diameter as the cord. The nerve cord was covered with a
Figure 7. Diagramatic representation of the essential components for x-ray exposure of the isolated abdominal nerve cord preparation and recording of neural activity between the second and third ganglia. Not drawn to scale.
layer of Van Harreveld solution and was rinsed periodically with chilled solution. The recording electrode, in both isolated and intact preparations, was placed between the second and third ganglia.

The electrical activity of the preparations was amplified and recorded with the use of the same equipment described in the section on the isolated chemoreceptors (see page 34). The activity of the preparations was recorded for 20 seconds before x-ray exposure, during, and 20 seconds post exposure.

**Exposure Methods.** All exposures were done with the Maxitron x-ray unit operated as described for the behavioral studies. The exposure rates used ranged from 10 to 35 R/s for durations of 20 to 30 seconds. Each preparation was entirely shielded with lead except for the sixth abdominal ganglion. As controls, the isolated preparations were exposed on the connective between the third and fourth ganglia, and the intact preparations were completely shielded and sham exposed to 35 R/s. This procedure was done to determine the degree of interference produced from electrical noise generated by the x-ray machine.

For tests of photoreactivity, the same light source was used as described in the ERG section, however the light passed through 60 cm of optic fibers. Figure 4 shows the light intensity in foot-candles at 2 cm from the tip of the optic fibers at various potentiometer settings. Light exposure duration was controlled manually with a switch. All the preparations were dark-adapted for 20 to 30 minutes prior to beginning tests with either light or x-rays.
Quantitation and Analysis of Data. Both isolated and intact nerve cord preparations exhibited spontaneous activity in varying degrees and the response to stimulation also varied, an observation made by others (Kennedy, 1963; Bruno and Kennedy, 1962; Prosser, 1933). In order to compare the responses of the preparations, it was necessary to normalize the data by comparison of the frequency of nerve impulses before, during, and after exposure to light or x-rays. The activity of the nerve cord was expressed as the spike frequency relative to that which occurred five seconds prior to x-ray or light exposure. A paired t-test was used to compare the activity of the preparations under exposure and control conditions.

The recorded electrical activity of the preparations was counted with a single channel pulse height analyzer (Ortec Model 406A) and scaler (Ortec model 430). The lower discriminator of the single channel analyzer was adjusted for each preparation above the noise level, in order to count the total activity, and counts were made in five second time bins before, during, and after light or x-ray exposure.
RESULTS

Radiation-Induced Behavioral Responses

Activity of the resting crayfish was initiated with x-ray exposure rates between 10 and 30 R/s. The animals appeared to be highly agitated and moved throughout the chamber. They would attempt to crawl up the walls of the chamber and pushed with their chelae against the acrylic plastic cover plate. The animals were active throughout the duration of the exposure and would either cease activity shortly after exposure or were active throughout the 60 second post-exposure period. This activity contrasted markedly with the activity pattern of the control animals. The controls generally remained in their original position throughout the sham exposure period. Those animals which displayed activity during the exposure period maintained this activity for brief periods and then settled down before the exposure ended. A few animals remained active throughout the observation period.

Analysis of the Motor Response to Sham-Exposure

Figure 8 is the pooled results of the controls and shows the proportion of animals that initiated motor activity for each ten-second interval during and after exposure. Less than ten percent exhibited any activity during each 10 second interval. Seventy-one percent of the animals did not respond at any time during the exposure period. The control groups from individual experiments
Figure 8. Distribution of respondents during sham-irradiation and one minute post irradiation for all tests (n=75). Less than ten percent exhibited any activity during each ten-second interval, and 71 percent of the animals did not respond at any time during the exposure period.
were compared against each other by Chi Square analysis to determine if there were any differences in the percent response during the sham-exposure. This analysis was done to determine if the response to sham-irradiation was random and not a function of events associated with the exposure. The percent of the animals that responded during sham-exposure from individual experiments ranged from 13 to 40 percent (Table 3). There was no significant difference between the groups.

Whole Body X-ray Exposure of Intact Crayfish

With an increase in radiation exposure rate, more of the animals displayed motor activity during the exposure period. The response was initiated earlier as the exposure rate was increased. The percent response during the period of exposure increased in almost a linear manner and reached 100 percent at an exposure rate of 30 R/s (Figure 9). At the highest intensity, 88 percent of the animals responded within 30 seconds after onset of exposure. Statistical analysis using the $X^2$ to compare the sham-irradiated control group with the experimental, indicated that at exposure rates of 10, 15, 20, and 30 R/s, a significantly ($p < 0.01$) higher proportion of the animals responded. The response to 5 R/s was not significant. It appears that the threshold for this response is between 5 and 10 R/s.

The average latent period within the sixty second exposure period decreased (Figure 10) with increased exposure rate. A "t"
<table>
<thead>
<tr>
<th>R/a</th>
<th>N</th>
<th>EXPERIMENTAL CONDITION</th>
<th>PERCENT RESPONSE DURING EXPOSURE PERIOD</th>
<th>LATENCY (SECONDS)</th>
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<tr>
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<td>25</td>
<td>INTACT WB 1</td>
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<td>28 ± 17</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>SHAM 2</td>
<td>33 (5/15)</td>
<td>30 ± 17</td>
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<tr>
<td>10</td>
<td>25</td>
<td>INTACT WB EXPOSURE</td>
<td>60 (15/25) (^4)</td>
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<tr>
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<td>15</td>
<td>SHAM</td>
<td>23 (2/15)</td>
<td>24 ± 15(^5)</td>
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<td>SHAM</td>
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<td>SHAM</td>
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<td>INTACT WB EXPOSURE</td>
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<td>SHAM</td>
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<td>OPTIMALECTORIZED SHAM</td>
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<tr>
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<td>22</td>
<td>POOLED INTACT CONTROLS</td>
<td>29 (22/75)</td>
<td>35 ± 16</td>
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</table>

(1) Whole Body. (2) Shielded Controls. (3) Not Significant. (4) p<0.01; \(^2\) test of differences between the sham control and the experimental group. (5) p<0.05; Student's \(t\) test of the differences between means when variances are unknown and unequal (Remington and Shork, 1970)
Figure 9. Percent response as a function of exposure rate (R/s). (○) Irradiated groups. (●) Controls (sham-irradiated). The dashed line represents the average response of the pooled control groups.
Figure 10. Latency for initiation of a motor response as a function of the exposure rate. (○) Irradiated groups. (●) Controls (sham irradiated). The dashed line represents the average response of the pooled control groups.
test analysis of the latencies, indicated that they were statistically different \( (p < 0.05) \) from the controls for all exposure rates except the 5 R/s exposure.

**Motor Response in Ophthalmectomized Crayfish**

**Whole Body Exposure.** The ophthalmectomized animals showed the same type of response to exposure as was described for the intact animals. These animals showed, however, a response with a latency of approximately nine seconds (Table 3). The response time of the blinded animals was significantly different \( (p < 0.01) \) from the response time of the intact animals subjected to the same exposure rate (30 R/s).

All ophthalmectomized animals responded within 30 seconds after onset of exposure, compared to 88 percent of the intact light-adapted animals subjected to the same exposure rate. Most of the blinded animals (77 percent) responded within the first ten seconds after onset of exposure (Figure 11A).

**Abdomen Exposure.** X-ray exposure to the abdomen initiated the same type of response as the whole body exposures of the intact and ophthalmectomized animals. Most of the animals initiated motor activity soon after onset of exposure, within 30 seconds (Figure 11b). However, the percent response within the 60-second exposure period was 81 percent, compared to 100 percent of the whole body exposures of the intact and ophthalmectomized animals (Table 3). The response latency was significantly greater \( (p < 0.05) \) than the whole body
Figure 11. Proportion of ophthalmectomized crayfish that responded in each ten-second interval from onset of x-ray exposure to one minute post exposure. Exposure rate was 30 R/s. (A) Animals subjected to whole body exposure (n=22). (B) Animals exposed to the abdomen (n=22). The black columns are the irradiated respondents and the open columns are controls.
exposure of the blinded animals and was comparable to that of the intact animals. Three ophthalmectomized animals did not initiate motor activity during the observation period. These animals appeared relatively sluggish and did not move actively about the exposure chamber when placed in it. Two of these animals died within two days after exposure and may have suffered from the effects of ophthalmectomy.

X-ray Exposure as an Aversive Stimulus

Crayfish, when given a choice of residence in a relatively darkened area as opposed to an illuminated area, prefer to reside in the darkened area. This photophobic response can be utilized to determine if an x-ray exposure is sufficiently noxious so as to cause the animal to go against his normal preference. If such was the case, the x-rays could be termed a stimulus for aversive behavior. A test was made to evaluate the motivational strength of x-ray exposure.

When the animals were initially placed in the exposure chamber, either in the illuminated or light-attenuated sections, they would explore the entire (35 x 15 x 10 cm) chamber. After five to ten minutes, the animals appeared to prefer the light-attenuated side. The residence time in the darkened end of the chamber (Table 4) by the sham-irradiated animals clearly indicated that the animals are photophobic. Over a five-minute period, the animals spent approximately 88 percent of the time in the darkened half of the
Table 4. Results of crayfish avoidance behavior during a 300 second x-ray exposure.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Residence time (Seconds)</th>
<th>Proportion of dark section (Dark/total)</th>
<th>Latency of escape from dark section (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-irradiated Control (n=20)</td>
<td>263 ± 32</td>
<td>.88</td>
<td>135 ± 81</td>
</tr>
<tr>
<td>X-ray Exposed (30 R/s; n=20)</td>
<td>131 ± 61</td>
<td>.44</td>
<td>64 ± 39</td>
</tr>
</tbody>
</table>

chamber. When the x-ray beam was turned on only the dark end was irradiated. During exposure to 30 R/s, the residence time in the dark end was significantly less (44 percent) (p < 0.01) than that of the controls. The escape time or time it took the crayfish to make the first crossing into the illuminated side after onset of exposure was significantly less (p < 0.01) (Table 4). After cessation of exposure, the animals were usually in the illuminated side, however when they crossed into the dark side they remained there for the remainder of the five minute post-exposure observation period.

During exposure, the subjects appeared to be agitated and exhibited increased motor activity. After the initial escape, the animals would cross over to the dark side for short periods and rapidly return to the illuminated side. It was also observed that during and after exposure, some animals would exhibit grooming and cleaning activity. These animals appeared to pick material from the surface of the carapace, around the eyes, and antennules with the chelae of the first two pair of walking legs.
Electroretinogram of the Isolated Compound Eye

The behavioral studies were designed to determine if the crayfish could detect x-rays via a non-visual route by the use of light-adapted animals and ophthalmectomized animals. The results indicated that a non-visual route could mediate detection. It was important, however, to establish and to confirm the visual system as a potential route of detection, since crayfish have been found to respond behaviorally to light intensities one-thousandth of those required to produce a measurable ERG (Bullock and Horridge, 1965).

The Light Evoked ERG

The approach was to characterize the bioelectric response of the eye to light and then compare the response to x-rays. The light evoked ERG was characterized by a large corneal negative deflection, the "on" wave, which occurred with the onset of the stimulus (Figure 12A). This initial wave was then followed by a positive phase which returned to base line. At termination of the stimulus a positive deflection occurred, the "off" wave. With increased duration of light stimulation the ERG amplitude increased in almost a linear fashion (Figure 12B) until it reached a maximum amplitude for the given stimulus intensity. A maximum ERG amplitude was obtained with a stimulus duration greater than 300 ms for 0.25 ft-c of illumination (Figure 13).
Figure 12. The light evoked ERG response. (A) "On" and "off" responses to a ten-second light stimulus at 0.25 ft-c of illumination. (B) The effect of increased stimulus duration as indicated above the trace in seconds. Calibration is 30 microvolts. Top trace is the stimulus marker; not functional in (B), and the bottom trace is the time marker in seconds.
Figure 13. The effect of increased stimulus duration on the light evoked ERG. 0.25 ft-c of illumination at the eye. The amplitudes were normalized against the maximum obtained at this level of illumination.
The X-ray Evoked ERG

The x-ray evoked ERG was similar to the light ERG. The onset of x-ray exposure produced a corneal negative "on" wave similar to the light "on" wave and on termination of the exposure, a positive "off" phase was evoked (Figure 14A). The response of the eye to increased stimulus duration was also similar (Figure 14B). Figure 15 shows the effect of an increased x-ray exposure duration on the amplitude of the ERG "on" wave at different exposure rates. The ERG amplitude reached a maximum for each exposure rate with exposure durations greater than 300 ms. This was similar to the response with light. The crayfish eye appears to have a rate limiting time constant of 300 ms at which a maximum ERG is produced for all exposure rates used. With exposure durations of 300 ms or less, there is a linear relationship between total exposure (R) and ERG amplitude (Figure 16). The linear relationship indicates that the ERG amplitude is dependent on total exposure and not exposure rate with exposure durations of 300 ms or less. With exposure durations greater than 300 ms the ERG increased in a linear fashion in relation to the logarithm of the exposure rate (Figure 17). This result is in agreement with the Weber-Fechner Law, which states that bioelectric responses of sensory receptors are proportional to the logarithm of the stimulus intensity (Guyton, 1961). In all cases the x-ray and light evoked ERG wave forms appeared similar.
Figure 14. The x-ray evoked ERG response. (A) "On" and "off" responses to a ten-second x-ray exposure of 7.0 R/s. (B) The effect of increased stimulus duration, as indicated below the trace in seconds. Calibration is 30 microvolts for (A) and 75 microvolts for (B). Top trace is the stimulus marker and the bottom trace is the time marker in seconds.
Figure 15. The ERG amplitude as a function of x-ray exposure rate and exposure duration. (○) 0.7 R/s. (●) 1.7 R/s (□) 3.3 R/s. (■) 5.0 R/s. (X) 7.0 R/s. N=5 crayfish eyes tested.
Figure 16. Amplitude of the x-ray evoked ERG as a function of total exposure (R/s x Duration). The relationship appears linear for all exposure rates with stimulus durations of 300 ms or less. (○) 0.7 R/s. (●) 1.7 R/s. (□) 3.3 R/s. (■) 5.0 R/s. (X) 7.0 R/s. N=5 crayfish eyes tested.
Figure 17. X-ray ERG amplitude as a function of the logarithm of the exposure rate at an exposure duration of 400 ms. N=5 crayfish eyes tested.
Dark-Adaptation

The time course for dark-adaptation after a flash of relatively bright light, was similar for both x-rays and light (Figure 18). However, the drop in ERG amplitude after the light flash (LF) was greater for light than x-rays. The reduction in amplitude was significant (p < 0.05) and suggested either that x-rays interact with some other component in the eye which does not detect the light, or that the accessory pigments in the eye absorb and block out the light stimulus.

Bioelectric Responses of Chemoreceptors

Response of the Antennule to Chemical Stimulation

Eight antennule preparations were tested with chemical solutions and x-ray exposure. Figure 19 shows the response of the antennule flagellum to administration of pond water and fish extract. The administration of fish extract produced a considerable increase in nerve impulse frequency. A variety of spike potentials were obtained and ranged from 60 to 100 microvolts in amplitude, and this activity was sustained as long as the stimulus was present. This finding agrees with that found in other crustaceans (Hodgson, 1958; Laverack, 1963). Rinses with pond water greatly reduced the activity. Fish extract appeared to be more stimulatory and produced spike potentials almost twice as large as those produced by glutamic acid. Glycine did not produce any significant response in the antennule preparation.
Figure 18. Dark-adaptation of the crayfish eye to x-rays (7.0 R/s) and light (0.1 ft-c). The amplitudes of the ERG responses to light and x-rays were equated before the onset of the light flash (LF=3.85 ft-c). (○) Light response. (●) X-ray response. N=5 crayfish eyes tested.
Figure 19. Spike frequency response of the medial branch of the antennule to stimulation with fish extract compared with stimulation by pond water as a control treatment. (A) Pond water. (B) Fish extract. Calibration for (A) and (B) is 60 microvolts and one second.
Response of Chemoreceptors of Leg to Chemical Stimulation

Recordings from the afferent sensory nerve bundles of the first walking leg also showed considerable spontaneous activity. Figure 20 shows the results of neural activity as it occurred in the walking leg with administration of pond water control and of glutamic acid. Glutamic acid consistently produced a greater degree of activity than did glycine and fish extract. The activity consisted mainly of two different size spikes; one approximately 30 microvolts and the other approximately 50 microvolts.

X-ray Exposure of Antennule and First Walking Leg Preparations

There was no apparent response to x-ray exposure. None of the preparations tested appeared to show any response during irradiation. Electrophysiological studies on marine crustaceans (Carcinas maenas, Portunus puber, and Homarus vulgaris) have shown that some chemoreceptors responded to chemical stimulation with a relatively long latent period, between ten and 50 seconds (Laverack, 1963). However, the preparations used in this study did not respond to x-rays even when the exposure was continued for one minute at 35 R/s and higher exposure rates.
Figure 20. Spike frequencies recorded from the afferent nerve trunk of the cheliped of the first walking leg after stimulation with glutamic acid (0.1 M) compared with stimulation by pond water as a control treatment. (A) Pond water. (B) Glutamic acid. Calibration for (A) and (B) is 60 microvolts and one second.
The Abdominal Nerve Cord and Sixth Ganglion

Bioelectric Response to Light Exposure

The response of the photosensitive neurons to illumination consisted of a gradual increase in the frequency of nerve impulses (Figure 21A). The spike potentials produced by the photosensitive neurons are distinctly different in amplitude (75-100 microvolts) from the endogenous non-photic background activity of the nerve cord (40-50 microvolts) (Figure 21B). Figure 22 is the results of eight preparations in which the total spike activity was counted in five second intervals and this activity was expressed as the spike activity relative to that which occurred in the five seconds prior to light stimulation. The response can be characterized by a prolonged latent period of two to five seconds, a continuous discharge, and a marked after-discharge at termination of illumination. The latency of the response decreased and the frequency of impulses increased with increased light intensity.

Bioelectric Response to X-ray Exposure

X-ray exposure of the isolated sixth abdominal ganglion produced a significant increase in spike activity (Figure 23), when exposed to 25 R/s or greater for at least ten seconds. The increase in spike potential frequency appeared to be due to stimulation of the photosensitive neurons only; however on closer inspection of the neural activity it was discovered that there was an increase
Figure 21. Light response of the sixth abdominal ganglion. Spike activity recorded from the isolated abdominal nerve cord with a ten second light exposure (0.25 ft-c) to the sixth ganglion. (A) Bottom trace is the stimulus marker. Calibration is 75 microvolts and one second. (B) The same preparation at a faster speed of the oscilloscope to show the characteristic spike potentials produced by the photosensitive neurons of the sixth ganglion. Calibration is 75 microvolts and 0.1 second.
Figure 22. Spike frequency of the abdominal nerve cord to illumination of the sixth abdominal ganglion, showing the relative change in spike frequency in five-second intervals with respect to the frequency during a five-second period prior to exposure. Exposure was ten-seconds in duration. Light intensity was 1.0 ft-c. Termination and onset of stimulus indicated by arrows. N=8 preparations.
Figure 23. X-ray elicited response of the sixth abdominal ganglion. The spike activity was recorded from the abdominal nerve cord upon exposure (30 R/s) of the sixth ganglion. (A) X-ray exposure. (B) Sham-irradiation. Calibration for (A) and (B) is 60 microvolts and one second.
in spikes of lower amplitude (40-50 microvolts). During light stimulation only an increase in activity of the photoreceptor neurons (75-100 microvolts) was elicited. This evidence indicates that possibly other interneurons in the crayfish CNS were stimulated and acted to mediate the detection of the x-ray exposure.

Figure 24 shows the relative spike frequencies per five-second intervals of eight isolated preparations exposed for 20 seconds to different exposure rates. The x-ray response appears to be similar to the light response in that there is a relatively long latency, a continuous discharge and a slight after discharge on termination of exposure. With an increase in exposure rate, the latency of an increase in spike activity became shorter. All of the results were compared to the control exposure, for statistical analysis. Significant increases in spike activity (p < 0.05) occurred after ten seconds of exposure to 25 R/s. Exposures of 30 and 35 R/s produced statistically significant increases (p < 0.05) after five seconds of exposure. Since sham-irradiation (shield attenuated at 35 R/s) did not show any significant increases in neural activity, electrical noise from the x-ray head could be ruled out. The increased activity was a biological effect of x-rays.

After determination that the isolated nerve cord preparation responded to x-irradiation, it was decided to make a determination in the in vivo preparation. The object of the study was to
Figure 24. Effect on the relative spike frequency of the isolated abdominal nerve cord when the cord and the sixth ganglion were exposed to different x-ray exposure rates. Onset and termination of the x-ray exposure is indicated by arrows. Controls were exposed at the connectives between the third and fourth ganglia at 35 R/s. N=8 preparations.
determine if a response to x-rays could be elicited and if the sensitivity would be greater, since the nerve cord and its ganglia would be relatively undamaged. Exposure durations were increased by ten seconds at those exposure rates which did not show any significant activity in the isolated preparation.

Figure 25 is the results with eight preparations. The response to x-irradiation was similar to that of the isolated preparation. A longer exposure at 20 R/s produced a steady increase in neural activity until the exposure was terminated; however the activity increase was not significant. Significant responses (p < 0.05) were obtained at 25, 30, and 35 R/s. It was observed that the isolated preparation showed a greater relative increase than the intact preparation. This was possibly due to the greater rate of endogenous activity in the latter preparation against which a response was compared.
Figure 25. Effect on the relative spike frequency of the abdominal nerve cord in vivo, when the sixth ganglion was exposed to increase x-ray exposure rates. Onset and termination of the x-ray exposure is indicated by arrows. Control was shielded and sham-irradiated at 35 R/s. N=8 preparations.
DISCUSSION

The main purpose of this study was to explore the possibility of non-visual detection of ionizing radiations in a freshwater crustacean, *Pacifastacus trowbridgii* (Stimpson). Previous studies by Martinsen and Kimeldorf (1971) and Kernek and Kimeldorf (1975), using carpenter ants (*Camponotus* sp) and red ghost shrimp (*Callianassa californiensis*) respectively, indicated that the detection of ionizing radiations could be mediated via olfactory or chemoreceptors. Partial body exposure of the red ghost shrimp induced significant avoidance behavior to x-rays, and indicated a possible radiation sensitive receptor in the abdomen. Subsequent electrophysiological work showed that bioelectric potentials could be induced in the swimmerette preparation with beta- and x-radiation. The nature or type of receptor involved was difficult to determine, although a chemoreceptor was implicated.

The crayfish, *Pacifastacus trowbridgii*, responded behaviorally to one minute x-ray exposures of 10 to 30 R/s. The subjects were light-adapted when exposed, thereby reducing the possibility of direct visual stimulation. Cues which might influence the animal's response during exposure could be the noise of the x-ray generator and possible fluorescence of the lucite plastic chamber. The fluorescence that could occur was presumably saturated by the illumination of the x-ray room with two 300 watt incandescent lamps so as to eliminate this as a factor. Controls for these experiments were shielded with lead during the exposure period.
so that the animals were subjected to all other factors of the exposure situation including noise and no significant effect was detected. The proportion of animals which displayed a behavioral response during the period of exposure increased with an increase in exposure rate and reached 100 percent response at 30 R/s. The latency for the arousal response was inversely related to the exposure rate. These relationships between exposure rate and response parameters are in agreement with behavioral work done with other arthropods (Terwilliger and Levy, 1964; Martinsen and Kimeldorf, 1971, 1972a & b) and with other invertebrates, tested with ionizing radiations (Dedrick and Kimeldorf, 1974; Kimeldorf and Fortner, 1971).

The whole body and partial body exposures of ophthalmectomyed crayfish were done to eliminate the possibility that visual pathways were involved in detection and to isolate a particular region of the body which might contain a radiation-sensitive receptor. Whole body exposure of these animals produced a significant degree of response. One hundred percent of these animals responded within 30 seconds from onset of exposure as compared with 81 percent for the intact animals at the same exposure rate (30 R/s). The average response time for the ophthalmectomyed animals (9 s) was significantly less than that of the intact animals (17 s) (Table 3). A similar phenomenon was observed by Kernek and Kimeldorf (1975) in ophthalmectomyed ghost shrimp. It appears that removal of the eyes in this crayfish, renders it more sensitive to detection of
radiation with respect to latency. No apparent difference in the response form was observed when compared to intact subjects, other than the latency. This may indicate that the intact and the blinded animals responded to the same mode of stimulation. Removal of the eyes may affect the manner in which sensory information is processed. Ophthalmectomy effectively eliminates all input along visual pathways and leaves the central processing free of input of possible interference from the visual system. It is difficult to provide direct evidence of the neural mechanism for this type of phenomenon. Sensory processing may be modified by a central filtering mechanism that processes information coming in from various sensory modalities (Manning, 1967). There is evidence in both vertebrates and invertebrates for central control of sensory inputs. Work by Anderson et al. (1974), Carpenter et al. (1963) and Galambos (1956) on mammalian vertebrates has shown that the CNS can exert efferent or centrifugal control over incoming sensory information in virtually all sensory modalities. In the crayfish, sensory input from tactile sensory neurons to interneurons has been found to be inhibited presynaptically (Kennedy et al., 1974; Krasne and Bryan, 1973) when the lateral giant fibers in the abdominal nerve cord are activated. Presynaptic inhibition also occurs in the mammalian spinal cord (Andersen et al., 1974; Carpenter et al., 1963) and is a process in which synaptic efficacy was reduced by prevention of the release of transmitter from presynaptic endings. In the crayfish, an interneuron reported to respond to
statocyst stimulation and a few hairs on the basal joint of the antennule, is completely depressed following visual stimulation (Wiersma, 1970). I have cited a few examples of possible mechanisms of sensory modification by the CNS that affect the manner in which sensory stimuli will produce a motor response. The removal of the eyes may allow other sensory pathways, which are being stimulated by x-rays, to have a greater influence on command interneurons for induction of motor activity.

Exposure of the abdomen initiated a behavioral response in a significant proportion of animals. This result indicated that a radiation sensitive receptor was located in the abdominal region. Shielding of the anterior portion of the animal with lead eliminated the possibility of direct stimulation of chemoreceptors in the antennules and the walking legs. The behavioral studies with whole body and partial body exposure of ophthalmectomized subjects definitely indicate that detection is not dependent on the principle visual organ. It is suggested that the sixth abdominal ganglion can mediate detection in the crayfish.

The crayfish will avoid a prolonged exposure to x-rays in a free choice situation. The response of other crustaceans has been variable and may depend on the particular species studied and the conditions of exposure. Axenfeld (1897a) observed that the isopod, Porcellius, moved from a shielded area toward the x-ray field, and indicated a phototropic response, since blinded animals did not respond. Terwilliger and Levy (1964) observed that the dark adapated
fiddler crab (*Uca pugilator*) exhibited a characteristic "off" response to both light and x-rays on termination of the stimulus. The response consisted of a cessation of motor activity, and removal of the eyes abolished the reaction. The dark-adapted and ophthalmectomized red ghost shrimp avoided an x-ray exposure of 52 R/s and indicated that x-rays are a noxious stimulus (Kernek and Kimeldorf, 1975). In the present study, the crayfish avoided x-rays in the presence of low illumination and the evidence indicated that x-ray exposure of a certain intensity constituted a noxious stimulus. The possibility of visual stimulation by fluorescence of the chamber was considered. The exposure chamber and the black paper used to reduce the light intensity were not observed to fluorescence during exposure by the writer under conditions of dark-adaptation.

The animals in the exposed side of the chamber became highly agitated and crossed over to the illuminated shielded side where activity was reduced. The animals also exhibited cleaning and grooming activity, a behavior that was observed when the animals were exposed to fish extract. This activity could have been due to the animal responding to stimulation of chemoreceptors by direct action of x-rays or from radiolytic products produced in the aqueous medium in which they were irradiated. The latter does not seem very likely, since the concentration of known products from the magnitude of exposures employed is not measurable.
The electrophysiological response that was first studied in the crayfish involved the electroretinogram (ERG). The ERG is a relatively slow, complex potential change generated by the photoreceptor layer of the visual sense organ in response to illumination. It is thought that the ERG represents a summation of generator potentials originating in the sensory cells themselves, along with contributions from postsynaptic structures in the eye. Arthropod eyes are divided into two categories (Kennedy, 1964; Bullock and Horridge, 1965): (1) "fast eyes" belonging generally to fast flying, diurnal insects (Apis, Caliphora), are characterized by a flicker fusion frequency over 200 per second, fast dark-adaptation, and yield ERGs with complex, diphasic wave forms; (2) "slow eyes" (cockroaches and most crustacea) show slow dark-adaptation and produce monophasic, corneal negative ERGs. The crayfish eyes fit the slow eye category in general.

The light evoked ERG is characterized by a corneal negative "on" response followed by an "off" response on termination of the stimulus. The ERG of the dark-adapted eye to light responds to increasing stimulus strength and duration up to some maximal time constant. These events and their relative magnitude are considered a consequence of the initial interaction of light with the photosensitive pigments (rhodopsins) located in the retinula cells of the compound eye.

The bioelectric response of the isolated crayfish eye was similar for both light and x-ray stimulation. The amplitude of the electroretinogram "on" response was found to increase with
increased stimulus duration for each exposure rate used until a maximum amplitude was attained with a stimulus duration greater than 300 ms. With stimulus durations of 300 ms or less, the ERG amplitude increased in relation to the total exposure for all exposure rates used. The eye appears to have a time constant of 300 ms for production of a maximum amplitude ERG for all exposure rates. Similar relationships were observed in the frog eye with light and x-ray exposure, by Bachofer and Wittry (1962 a & b) and Dawson (1965). With stimulus durations greater than 300 ms the ERG amplitude increased in relation to the logarithm of the exposure rate and this was in agreement with the Weber-Fechner Law. Similarity of the bioelectric response of the eye to both light and x-rays has been found in other arthropods (Jordan and Kimeldorf, 1971; Martinsen and Kimeldorf, 1972b; Smith and Kimeldorf, 1964) and indicated that the interaction of light and x-rays with the photosensitive pigments of the eye was similar.

The time course for dark-adaptation in the isolated eye was found to be similar for both x-rays and light. The reduction in ERG amplitude after the isolated eye was given a 500 ms light exposure (3.85 ft-c of illuminance) was significantly greater for light than for x-ray stimulation. Smith and Kimeldorf (1964) observed a similar phenomenon in studies of the dark-adaptation process in Noctuid moths. Histological examination of the moth eye after three minutes of light exposure showed that accessory distal pigments migrated and would interfere with the transmission
of light to the photosensitive elements of the eyes. Ionizing radiation, however, is not absorbed by the accessory pigments and the bioelectric response reflects the regeneration of the photosensitive pigments involved in the visual process. The difference observed in the crayfish eye during dark-adaptation may be attributable to migration of accessory distal pigments as a result of illumination of the eye prior to testing for dark-adaptation. This effect was eliminated, in the case of the moth eye, when the light flash was considerably shortened to a pulse of approximately 20 ms. The duration of the light flash used in the present study on the crayfish eye was 500 ms and much less intense than that used for the moth (175 ft-c). The results would suggest that pigment migration is a rapid process, since differences in ERG amplitude could be observed within at least 30 seconds after the light flash. Movements of the distal pigments are controlled by a hormone of the sinus gland located in the eye stalk. This, in turn, is indirectly influenced by light shining into the eye. The hormone action would have to be relatively fast to produce the observed effect.

An alternative possibility which would account for the observed difference during dark-adaptation would be that x-rays and light interact with different visual pigments. Two visual pigments have been identified in crayfish eyes (Kennedy, 1964). Spectral sensitivity maxima have been found to occur at 508 and 562 nm in *Orconectes virilis* (Kennedy, 1964) and at 570 nm in *Procambarus clarkii* (Bruno
and Kennedy, 1962). After the initial light flash in the cited experiments, the light intensity was reduced from 3.85 ft-c to approximately 0.1 ft-c with a potentiometer. This procedure produces a spectral shift in the light source toward the longer wave lengths. If P. trowbridgii has two visual pigments, then it is possible that the light may interact to a greater degree with the pigment that has a spectral sensitivity toward the red region, whereas x-rays interact with both pigments.

Early behavioral studies with pieces of meat and solutions from macerated meat, indicated that decapod crustaceans possess chemoreceptors at various sites. Direct stimulation of the antennules, antennae, mouth parts and tips of the chelae and the chelipeds on the walking legs, produced behavioral excitation, especially of the stimulated structure (Holmes and Homuth, 1910). It was concluded that the "seat of smell", in crayfish, was located in the antennules.

Electrophysiological studies by Hodgson (1958), showed that chemoreceptors on the medial branch of the antennule, the chelae, and the chelipeds of the first two pair of walking legs in the crayfish responded to solutions of glycine and glutamic acid. The lateral branch of the antennule showed little chemoreceptor activity. Electrophysiological investigations of chemoreception in related crustaceans (Homarus sp., Panulirus sp., Carcinus sp.) also identified chemoreceptors on the antennules and the walking legs (Case and Gwilliam, 1961; Case, 1964; Laverack, 1963, 1964;
Levandowsky and Hodgson, 1965). Other investigators have indicated that chemoreceptors may be located throughout the animal's body (Bullock and Horridge, 1965).

The medial branch of the antennule and the first walking leg were selected for this study because of their relatively high concentration of chemoreceptors. Studies on the detection of ionizing radiations have implicated olfactory and chemoreception in certain vertebrates (Cooper and Kimeldorf, 1966) and arthropods (Martinsen and Kimeldorf, 1971; Kernek and Kimeldorf, 1975) as primary routes of detection.

Olfactory receptors in the medial branch of the antennule and taste receptors in the chelifeds of the first pair of walking legs did not respond significantly to x-ray exposure in the crayfish. Even prolonged exposure and at exposure rates above 30 R/s, at which 100 percent response was obtained, produced no bioelectric response. An analysis similar to that done with the ganglion preparation, in which spike frequency data from several preparations was quantified, did not show a significant shift with x-rays. The difference in radiation-sensitivity of chemoreceptors shown by the crayfish and other arthropods and vertebrates, possibly reflects basic differences in specialization which have evolved in these animals. Insects, in particular the ants, have developed highly sensitive chemoreceptor systems which allow them to communicate with each other (Prosser, 1973). The red ghost shrimp has evolved a highly specialized existence, living in mud or sand burrows. These animals have poorly developed eyes and may have a highly
developed chemosensory system which allows the animals to detect changes in their environment. The crayfish may not have evolved chemosensory systems to the extent that other arthropods have. This may be reflected in the insensitivity of its chemosensory system to ionizing radiation.

The initial discovery of the photosensitivity of the sixth abdominal ganglion was made by Prosser (1933). Studies by Kennedy (1963) determined that there are only two photosensitive neurons in the sixth ganglion and that they are complex, secondary interneurons, that receive inputs from tactile receptors in the tail. Spectral sensitivity studies by Bruno and Kennedy (1962) in the crayfish, Procambarus clarkii, have shown that the spectral sensitivity peak occurs at 500 nm. The sensitivity curve shape agrees with the absorption curves of previously extracted crustacean rhodopsins (Kennedy and Bruno, 1961; Fernandez, 1973). Studies of leg movements in the crayfish by Welsh (1934) have shown that a significantly greater leg activity resulted when the caudal ganglion was exposed to light than with exposure of the eyes.

The photoreceptor nature of neurons within the sixth abdominal ganglion suggested another possible route for detection of ionizing radiation. The fact that the dark-adapted eye responded to ionizing radiation suggested that the mechanism of interaction with a photosensitive pigment may be involved.

X-irradiation of the sixth abdominal ganglion in both the isolated and intact nerve cord initiated activity of the photosensitive neurons and other neural elements in the ganglion. The
response of the photosensitive neurons was distinguished from background noise by the characteristic spike potentials produced, which were relatively large. In both the intact and isolated nerve cord preparations a significant increase in spike frequency occurred after 10 seconds at 25 R/s and the latency decreased to within 5 seconds at 30 and 35 R/s. The intact preparation did show an increase at 20 R/s, however this was not significant when compared to the sham irradiated control. The latencies for the bioelectric responses and for the behavioral arousal observed in whole body exposure of ophthalmectomized animals are in close agreement and would indicate that detection of ionizing radiation may consist of activation of the crayfish CNS. Interaction of x-rays with a photosensitive pigment in the photosensitive neurons of the caudal ganglion is possibly one mechanism of action.

Activation of other neurons in the caudal ganglion possibly occurs at the level of the synapse. X-rays may initiate depolarization at (1) the post synaptic membrane, which is a specialized chemical receptor, (2) at the presynaptic membrane and induce transmitter release, or (3) possibly both effects are produced. Ionizing radiations have been found to produce transient increases in membrane permeability to sodium and potassium, however, relatively high exposures and exposure rates were necessary (Gaffey, 1962; Darden, 1960). It is possible that x-irradiation of the presynaptic membrane produces a release of calcium, which has been found to
be necessary for synaptic transmitter release (Katz and Miledi, 1964; Del Castillo and Engbaek, 1954). Membrane bound calcium has been found to be released by x-radiation and sulfhydryl reagents from red blood cell membranes (Tolberg and Macey, 1972). The calcium release in conjunction with potassium, occurred with exposure rates of 6.6 to 16.6 R/s, which is within the range used on the crayfish preparations. Brinkman (1962) discussed cases where irradiation of the head in mammals produced a release of serotonin, histamine and epinephrine. These products were detected in the urine of the animals.

There have not been any cases cited in the literature of direct stimulation of the CNS in invertebrates. Sublethal whole-body and head exposures in mammalian vertebrates have produced alterations in spontaneous and evoked electrical activity in the brain (Arnold et al., 1954; Monier and Krupp, 1962; Haley, 1962; Cooper and Kimeldorf, 1964). Exposures between 200 and 400 R produced transitory, reversible alteration in the rabbit brain (Monier and Krupp, 1962) and cat brain (Haley, 1962). In the case of the cat, whole body irradiation produced the CNS changes and possibly peripheral mechanisms were involved. In the rabbit, localized irradiation of the head was followed by hyperactive motor behavior and activation of the EEG with desynchronization. The reversibility of the electroencephalographic symptoms after irradiation suggests an action of ionizing radiation on neuronal and synaptic activities. The effect observed by Hug and Schliep (1963) in which the intact leech nerve-muscle preparation was
more sensitive than the muscle alone to x-rays, indicated the possibility of neural or synaptic activation in invertebrates.
CONCLUSIONS

1. X-ray exposure induced an immediate motor arousal in the crayfish, *Pacifastacus trowbridgii* (Stimpson). The animals responded to exposure rates of 10 to 30 R/s. The latency of response was inversely, and the proportion of respondents was directly, proportional to the exposure rate. The threshold exposure rate was between 5 and 10 R/s and 100 percent of the subjects responded to 30 R/s.

2. Whole body exposure of ophthalmectomized crayfish at 30 R/s induced a similar response as in intact animals; however 100 percent of the subjects responded within 30 seconds of onset of exposure, compared to 81 percent for intact animals. The latency of response was significantly less in blinded animals. Partial body exposure of ophthalmectomized animals induced a significant proportion of animals to respond, and indicated a radiation-sensitive receptor was localized in the abdomen.

3. Electrophysiological studies of the dark-adapted crayfish eye suggested that the visual system could act as a route of detection of ionizing radiation. The electroretinogram is similar for both light and x-rays, and indicated that light and x-rays react with the photosensitive pigments in the eye in the same manner. Electroretinograms were elicited with x-ray exposure of 0.7 to 7 R/s. The amplitude increased with increased stimulus duration and reached a maximum for each exposure rate with a pulse greater than 300 ms. With stimulus durations greater than 300 ms
the ERG amplitude increased in relation to the logarithm of the stimulus intensity of exposure rate. The ERG increased linearly with total exposure with exposures of 300 ms and less.

4. The time course for maximal dark-adaptation to both x-rays and light appears to occur in approximately nine seconds after a light flash of 3.85 ft-c of illuminance. The initial difference in ERG amplitude produced by light and x-rays can be attributed to absorption of light by migrating accessory pigments or by differential interaction of the light stimulus with photopigments in the compound eye.

5. Chemoreceptors located in the medial branch of the antennule and the cheliped of the first walking leg did not respond to x-ray exposure. Exposure rates greater than 30 R/s and one minute in duration did not produce any significant effect in these preparations. The presence of chemoreceptors was indicated, however, by responses elicited by administration of glutamic acid, glycine, and fish extract.

6. Bioelectric responses were recorded from the dark-adapted, isolated and intact abdominal nerve cord during x-ray exposure to the sixth abdominal ganglion. Significant increases in nerve impulse frequency occurred after 10 seconds of exposure at 25 R/s and after five seconds at 30 R/s and 35 R/s. The latency for a significant bioelectric response was within the range of the latency for the motor response.
7. The response to x-rays in the sixth ganglion appears to involve more than simple photoreception. During x-ray exposure there was an increase in the frequency of spike potentials of smaller amplitude (40-50 microvolts) than those produced by the photosensitive neurons (75-100 microvolts). This indicated that neural elements other than the photosensitive neurons were being activated.


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