

1964

The behavioral responses of the fiddler crab, UCA PUGILATOR, to ionizing irradiation

<https://hdl.handle.net/2144/32857>

"Downloaded from OpenBU. Boston University's institutional repository."

BOSTON UNIVERSITY

GRADUATE SCHOOL

Thesis

THE BEHAVIORAL RESPONSES OF THE FIDDLER CRAB,
UCA PUGILATOR, TO IONIZING IRRADIATION

BY

Robert Terwilliger

(A.B. Bowdoin College, 1962)

Submitted in partial fulfillment of the requirements
for the degree of Master of Arts, 1964.

AM
1964
+
c.1

Approved by

First Reader .. *Charles K. Ferguson*
Professor of Biology

Second Reader .. *Frank A. Belamirich*
Professor of Biology

Acknowledgement

I would like to thank Dr. Charles Levy for his continual guidance and suggestions in the preparation of this thesis. I particularly appreciate the extra time he spent with me when it was inconvenient for him to do so and his encouragement when problems arose.

I would like to thank Dr. Frank Belamarich for his very helpful ideas and particularly for his time spent in advising me on the first draft.

I would further like to thank John Degelman for his ideas and help in the construction of the recording device, and Jim Menzoian for the time he spent assisting me with the subjective observations.

Topical Outline

Chapter	Page
I. Introduction.....	1
A. Properties of Photoreceptors.....	1
B. Early History.....	2
C. Comparison of X-ray and Light Phosphenes...	4
D. Possible Mechanisms.....	6
E. Behavior.....	9
1. Vertebrate.....	10
2. Invertebrate.....	12
F. Purpose.....	17
II. Method and Materials.....	18
A. Animals.....	18
B. Radiation.....	18
C. Dosimetry.....	18
D. Recording.....	18
E. Analysis of Data.....	19
F. Subjective Observation of behavior.....	19
G. Experimental Design.....	19
III. Results.....	21
A. Gross Behavioral responses of <u>Uca pugilator</u> to whole body x-irradiation and light.....	21
B. The effects of whole body x-irradiation and light on eyestalkless <u>Uca pugilator</u>	22
C. The effects of dose rate on the response of <u>Uca pugilator</u> to whole body irradiation....	22

Chapter	Page
IV. Discussion.....	25
A. Possible sites of sensitivity to x-rays.....	25
1. Indirect activation through endocrine substances.....	25
2. Direct activation of neural structures..	26
3. Photoreceptors.....	26
B. Possible Mechanism.....	27
C. Dose rate dependency.....	28
V. Conclusion.....	29

List of Illustrations

	Page
I. Table I.....	23
II. Figure I.....	24

Introduction

Most sensory receptors are especially sensitive to one form of energy, the adequate stimulus. This stimulus not only elicits a response, but it is the form of energy to which the threshold of the receptor is the lowest. Müller's law of specific nerve energies states that once the stimulus energy is transduced, even if the receptor is activated by a form of energy other than the adequate stimulus, the subjective response is always the same.

Photoreceptors are sensitive to a narrow band of the electromagnetic spectrum from a wavelength (λ) of 327 m μ to a λ of 723 m μ . Some invertebrate photoreceptors, such as those of bees, are able to transcend this limitation by perceiving the shorter wavelengths of the ultraviolet. Not all wavelengths of light are equally effective stimuli, and the wavelength will influence the intensity of light necessary to evoke a sensation. Compared to other receptors, the photoreceptors exhibit extreme sensitivity to their particular form of stimulation, nearly attaining the lowest theoretical limit of sensitivity, one quantum of light. (37)

The possibility of electromagnetic waves with a shorter wavelength than light being able to produce a response via the photoreceptors does not violate any classical law. Although it is not the adequate stimulus, these shorter wavelengths could elicit a response either by the same mechanism as light, a photochemical stereoisomerization, or by an en-

tirely different mechanism. If the energy were transduced, the response would be a visual response as required by the law of specific nerve energies. Any behavior normally associated with exposure to light should manifest itself when this other form of stimulus is employed.

With the discovery of x-rays in 1895, Roentgen, noticing that these rays, like light, caused a photographic film to darken, suggested that x-rays might also elicit a visual response through activation of visual receptors in a manner similar to that of light. Although his initial attempts to demonstrate this were unsuccessful, an interest was aroused in this problem. At first, the failure was ascribed to the opacity of the ocular media. However, when the experiments were performed on aphakic (lensless) subjects or when the interference of the ocular media was further reduced by irradiating the retina directly through the sclera, no response was noted. The failure of these early investigators was attributed to an insufficient penetrance of the x-rays due to poor x-ray generators. A few years later, Roentgen was able to overcome his initial failure by evoking a light sensation with x-rays. (27) Brandes and Dorn in 1896 were among the first investigators to report an x-ray caused light sensation produced in a blindfolded aphakic, and numerous reports apparently confirming their results subsequently emerged. (27) Many other investigators, working at this time with blind and normal subjects, were able to pro-

duce the light sensation with x-rays although the actual mechanism was subject to argument. Hilmstedt and Nagel, 1901, were able to measure the action current between the fundus and cornea of an excised frog's eye. When the retina was stimulated by x-rays, an action potential was produced similar to that evoked when the stimulus was light. They also noted that the action potential was weaker when the animal was light adapted and became stronger with dark adaptation. The action current which they described was of the b wave type. Since this action current is now thought to be associated with rod activity, it would appear that the locus of x-ray stimulation is at the rod level. The same investigators also worked with frogs, owls, chickens, turtles and pigeons. Although the owl and the frog responded well, the chicken showed no response to x-irradiation. (27) The greater number of rods in the frog's and owl's eye may explain these results. It was also apparent early in the history of this phenomenon that the threshold of stimulus intensity varied among different subjects. By 1902, the disagreements had been settled, and x-ray perception by photoreceptors was confirmed. Most experiments were discontinued after 1906 because of the deleterious effects of x-rays on living tissues.

After a dormant period of close to thirty years, many investigators renewed attempts to discern similarities between the visual sensation caused by light and x-rays.

Early workers had noticed that the eye had to remain in the dark or undergo dark adaptation before the x-ray could be perceived. Dark adaptation is also necessary when weak light is used as a stimulus. Dawson and Smith, working with the horseshoe crab, Limulus polyphemus, noted the effect of low level irradiation on the threshold shift in the visual receptors. When the animals were subjected to irradiation, there was a marked visual response which was most pronounced at the lower dosage levels and which was cancelled by adaptation to light. They were able to characterize tentatively the mechanism as photochemical. (12) A pupillary response similar to that induced by light was produced by x-rays. A visual response to light will remain on the retina after the source of stimulus is removed. X-rays also exhibited this effect. There is a critical area which must be stimulated on the retina in order to produce a response to light. This requirement was characteristic for x-rays. (27) Bornschein, Pape, and Zakowsky noticed that the threshold dose was constant for low durations but then increased as the duration increased. This reciprocity of duration and intensity of stimulus was also noted for light. (8) The similarity of an increase in light threshold with age to the increase in x-ray threshold was also evident. (27)

The light phosphene, the subjective visual sensation experienced when the optic nerve is stimulated by light, has different characteristics than the x-ray phosphene, a

fact which presents difficulties when an explanation of the mechanism of x-ray stimulation is attempted. When human subjects underwent whole body irradiation, they reported a variety of colors and general appearances of the x-ray phosphene. All descriptions were quite different from the constant color sensation produced by light. The phosphene of x-rays differed depending on stimulus intensity, whereas that for light did not. Through examination of x-ray shadows on the retina of frogs and other animals, the following conclusions were reached. (27) It was noticed that the ocular media did not refract x-rays and that x-rays produced an area of excitation when they intersected the retina. However, the projection, associated with the light phosphene, is the same as that with x-rays, an observation which should hold according to the law of specific nerve energies. Lipetz (27) felt that, since the x-rays were not refracted by the media of the eye, the form of the image on the retina was due to a silhouette of the object perceived. The periphery of the retina exhibits a higher sensitivity to x-rays, a fact which further supports the hypothesis that the rods are the receptors most sensitive to x-rays. (27)

These preliminary studies and comparisons led to numerous attempts to confirm these findings as well as to explain the behavioral responses which resulted. The main controversy was whether radiation was perceived through visual receptors as supported by Hug or whether the vegetative nervous system was

the vehicle, a view supported by numerous Russian workers. (25) They felt that only the visible spectrum could be sensed by photoreceptors and that evolutionary development had not provided an x-ray receptor. Hug suggested that perhaps there is a latent ability which still persists. (25)

Lipetz, one of the main investigators working on x-ray perception, had attempted to design experiments which might elucidate the physiological mechanism involved in this phenomenon. His working hypothesis was that since light energizes and bleaches visual purple, x-rays should also work by the same mechanism. He discarded the possibility of the central nervous system as a receptor to x-rays since curarized frogs responded in a manner similar to pithed frogs. The optic nerve ganglion cell of the frog retina was utilized to produce an electroretinogram (ERG). The ERG which was recorded was examined closely, and the pattern of response showed similarities to a light evoked ERG. When the frog retina was stimulated, there was a burst of electrical impulses from the ganglion cell layer which signified an on response. Then there was a continuous discharge of impulses followed by another burst of impulses marking the off response. This pattern of impulses was of the same wave form and had the same discharge rate as an ERG of light. The type of response was also similar. He noted that light and x-rays effect the retina similarly in that exposure to either one reduces the responsivity to both. In attempting to determine

threshold x-ray stimulus, he used the off response due to its particular clarity on the ERG and concluded that more energy had to be absorbed from x-rays than from light for a threshold response. Lipetz was able to derive some conclusive similarities hitherto presented without proof. Both x-rays and light have the same type of discharge, on-off response, with the same relative responsivities for the different types of discharge. If either one is used as a stimulus, the responsivity to both is reduced, and the responsivity to both is increased during subsequent non-stimulation. When the test time is increased between stimuli, there is a lowering of threshold to that stimulus. The latency of response was the same for both. In an examination of duration of stimulus, Lipetz found that there was critical duration less than which a constant dose was needed to produce a threshold response. This duration was six seconds for light and two seconds for x-rays. He also found that if he applied frequent, near threshold x-irradiation, there was a cumulative temporary rise in x-ray threshold with respect to light threshold, an effect not evoked by light. When a near threshold x-ray stimulus was used, there was an immediate temporary rise in the light threshold. This effect was also not observed with a light stimulus. Again, if he applied a threshold x-ray stimulus, the x-ray threshold was raised five times more than if he applied a threshold light stimulation and observed the corresponding increase in light threshold. Finally, he noticed

that x-irradiation temporarily reduced the voltage of the ganglion cell discharge, and light did not. He suggested that x-rays tend to desensitize the retina and that the few differences noted above could be because of the normal injurious effects of x-rays. (28) In 1941, Kektcheew, experimenting on humans, noticed that there was a change in threshold of achromic vision after total or partial body irradiation. After a fifty minute dark adaptation period, an increase in sensitivity to x-rays was observed. A sharp decrease in sensitivity followed, corresponding directly to the number of tests performed. There was a significant drop in the threshold for light when any portion of the body was exposed to irradiation, and Kektcheew was able to produce a rise in threshold at times, depending on the state of the individual tested. The implication here as to the mechanism involved is that the vegetative nervous system responds, and this effects the achromic visual threshold. (23) In 1959, Lipetz was able to produce observable effects at the organ level, the eye, with as low as $\frac{1}{2}$ mr.. This result suggests that radiation is able to trigger a sequence of amplifying processes which lead to the response. (29) Veninga also worked with the frog's eye and compared light induced ERG's with those produced by x-rays. There was a noticeable fatigue effect if the interval between stimuli was less than one minute. He also showed how the ERG evoked by light could be attenuated by the x-rays, and how the converse

could occur. The off response produced a peak on the ERG with a lower amplitude than the on response in many of his experiments. (41) Recently, Bachhofer and Wittry, also working with Rana pipiens, noticed that the x-ray evoked ERG was similar to the ERG produced by light, but although the on responses correlated, the off responses did not. The type of off response recorded depended directly on the strength and duration of the stimulus. (4) In another experiment they noticed that the retina was able to recover completely from short flashes of x-rays at different frequencies of stimulation. When subjected to light, the visual receptors evoked no ERG response to x-rays. After exposure to x-rays, the light ERG is reduced. They concluded that the interaction between photoreceptors and x-rays is different from that between photoreceptors and light. (3) Similar experiments were performed on an invertebrate, the cockroach, Blaberus giganteus, and a marked effect on the amplitude and frequency of the ERG due to large doses of x-rays was observed. (5)

The mechanisms by which x-rays are able to evoke a response are still not clear. The differences between the character of light perception and that of x-ray perception seem to suggest a different mechanism. However, regardless of how the impulse is generated, the subjective response will always be a visual sensation if the optic nerve is stimulated. Thus, any behavioral response induced by light should also result from x-irradiation. These responses have been found

to exist in the form of an acknowledgement of a noxious stimuli, an effect of conditioning and a general change in activity.

One of the earliest behavioral experiments with vertebrates involving radiation exposure was done by Chaluppecky, 1897. When irradiation was administered to a dark adapted dog for one hour, there was no behavioral response noted although the retina showed pathological effects. This failure can probably be attributed to the low intensity x-ray generator. (11) Some of the more recent investigations included a conditioning experiment where the taste perception in rats was influenced by x-irradiation. When the rats associated saccharin flavored water, which before application of x-rays was favored, with the irradiation, they tended to show a marked aversion for this fluid. (15) Similar experiments utilizing cats and rats produced avoidance of a previously preferred stimulus. (24) These experiments illustrate the effectiveness of ionizing radiation acting as an unconditional stimulus in the behavior of animals. Further studies were conducted with rats by Overall and Brown, using a shuttlebox arrangement to measure the response to irradiation. They concluded that x-rays have an immediate effect on rats and are perceived as a noxious stimulus from which escape is sought. They also suggested that the physiological effects produced by incident radiation overshadowed the normally noxious effects of brightness and glare. The results were obtained

with a surprisingly low dose rate of one r/min. (35) Later some more objective tests showed the observed effects to be quite moderate. (30) Behavioral studies were carried out on male albino rats which had been trained to respond to a food stimulus by pressing a lever. Randomly divided groups were then subjected to varying amounts of irradiation as well as varying numbers of exposures. There was a change in response corresponding to both variables, the largest variable being the cumulative dose rate received by the animal. (9) A more recent experiment employing the same type of lever pressing device, illustrated the ability of animals to recognize an x-ray clue in their environment, yet no mechanism was suggested. (13) Andrews and Cameron noticed that there was an immediate response in mice in the form of an avoidance behavior to high energy radiation, 50 r/min. and above. In this experiment ventilation was controlled in an attempt to eliminate the problem of ozones and oxides of nitrogen which might be perceived via sensory receptors other than visual. Equilibrium concentrations of gaseous radiation products should be attained rapidly, and recognition should be prompt. Since the applied dosage was constant and the time at which avoidance was evident varied, they argued against secondary stimulation. (1) When rats, guinea pigs and hamsters were subjected to whole body irradiation, a depression of diffuse activity was recorded by a spring suspended unit. (10) With low intensity x-rays, Hunt and Kimeldorf were able to waken

a sleeping rat after a short latent period of a few seconds.

(22)

Some other vertebrates demonstrated different behavioral responses to ionizing radiation. A high intensity beam initiated behavioral responses in certain species of fish. The subjects increased a forward darting and backward swimming movement and accelerated movement of the gills. X-rays had an effect on the acquisition and retention of conditioning, and a general hyperactivity was noted during irradiation. Unfortunately, the chemical changes in the water were not controlled during this experiment. (38) Krebs, working with the red-eared turtle, Pseudemys scripta elegans, noticed positive responses to irradiation. The turtle first raised its head as if to localize the stimulus and then moved suddenly out of the beam. Often the turtle would brush its head with its feet as if to remove an irritant. As the number of exposures was increased, the reaction time decreased, passed through a broad minimum, and then sharply increased. This description of the stimulus is similar to Baylor and Smith's findings with Limulus polyphemus. Using a high dose rate, 2900r/sec., Krebs was unable to find any occurrence of bleaching after histopathological investigations of the turtle's retina. The reason for the high threshold in this particular animal was attributed to the activation of an all cone receptor. (25)

Some early studies of invertebrate behavior were carried

out by Axenfeld in 1896. He worked with some insects, coleopterans, dipterans, and hymenopterans, and with the crustacean, Porcellius. His apparatus consisted of a box, half of wood, half of lead, from which all light was excluded. Upon x-irradiation, the animals showed a marked preference for the wooden side of the box, a response which he attributed to photophilia. When the animals were blinded, they no longer responded, and Axenfeld concluded that a visual sensation had been produced. (2)

Later workers, equipped with more powerful x-ray generators, continued work in this area. In response to a high intensity beam of 38,000 r/min., behavioral activity was noticed in the mosquito Culex. The movements of the larvae were accelerated after the onset of irradiation. (16)

Olivard and Hungate noted that Drosophila would respond to ionizing radiation. (33) Baylor and Smith found that in the presence of red light, Daphnia, when irradiated, would move downward. This response indicates a preferential stimulation of the nauplius eye. Fluorescence as well as heat was removed as a cause of these movements. Another interesting result of their experiments was that redox poisoning compounds were able to evoke the same response. (6) Hug filmed the responses of many invertebrates. In three species of snail, he observed the retraction of tentacles at the onset and throughout the application of irradiation, yet, when the tentacles were shielded, no such response occurred. Snails

are known to retract their tentacles when a mechanical, chemical, or optical stimulus is applied. There was a noticeable latency, and the speed of response was dependent on the strength of stimulus. While a minimum dose rate was necessary to elicit a response, it varied with the species tested. As the dose rate was increased, the latency was reduced, and the reflex was more pronounced. There was a minimum exposure time at threshold dose rate, yet this duration depends on many variables such as season, temperature, etc. Hug also observed responses in the leech, Hirudo medicinalis. At low dose rates, .75-2.5 r/sec., the animal exhibited peristaltic contractions of its body, and at higher dose rates, it detached its sucker and moved violently from the radiation beam. In an attempt to determine the mechanisms involved in the reactions of the leech, he used isolated nerve-muscle preparations of this animal. Upon irradiation, changes in tone and contractility were noted. A sea urchin, Echinus miliaris, will retract its tube feet from the surface of the irradiated water, the reaction again dependent on dose rate. Hug also studied the radiation responses of the barnacle, Balanus balanoides, and observed the evocation of rhythmic motions, the opening of the cover, and grasping movements with the cerripecta. Continuation of the exposure at 10 r/sec. slowed down the movements and after 6-7 seconds caused the animal to close its shell. A short time later, the movements, slowed a bit, would resume. The reaction

seemed to be dependent on the initiation of irradiation or a rapid increase in dose rate. Other arthropods showing radiation behavioral responses were flies, beetles, spiders, butterflies, and caterpillars. All of the tested animals exhibited a sensitivity to light, and similar reactions could be induced with x-rays as the stimulus. Sometimes, however, it was not because of an increase but rather a decrease in light that these reactions occur. Both Helix pomatia and Balanus react to a decrease in light with the characteristic shadow reflex. Experimenting with ants, Hug observed numerous reactions. The ants suspended the normal actions of licking their companions and showed agitation, exaggerated cleaning of their antennae as well as defensive attitudes. When given the option of a lead shielded portion of their container, the animals soon occupied this area. Again, the dose rate and latency varied with the species tested. When Camponotus were subjected to a dose rate of 70 r/sec., all animals were under the lead within 75 seconds. Hug attributes some of the ant defense reactions to chemical effects on the organs of taste and smell. In addition to his behavioral studies, Hug did some pertinent work with isolated mammalian organs in an attempt to induce immediate responses. He was able to change the tonus and peristalsis in an isolated rabbit intestine. A threshold dose was needed in this case also. He irradiated blood vessels and noticed a lowering in perfusion rate. Referring to the work of

previous investigators, Hug suggested that the locus of stimulation was parasympathetic at the enteric ganglion and indicated the possibility that x-rays may elicit an instantaneous reaction by acting directly on cells and organs other than the photoreceptors. There are tissues, nerve elements such as the abdominal ganglion of lobsters and worms, which are sensitive to light and which may also be sensitive to irradiation. (20) Born, stimulated by the work of Hug, experimented with pulmonate snails and observed the contraction of the mantle cavity in response to ionizing radiations, a response similar to the tentacle retraction of Hug's snails. Since the outer margin of the mantle cavity is the area most sensitive to light for most snails, Born also suspected a visual response. (7) Smith, Kimeldorf, and Hunt recently recorded the motor response of a moth to low intensity irradiation. After the animals were first dark adapted, they were subjected to a dose rate of .01-1.5 r/sec., and a flight response was observed. The index of response was wingbeat frequency which could be initiated in resting moths or augmented in active moths with a latency of less than one second. Employing a ceramic crystalline piezoelectric transducer to record the response, they varied the dose rate and concluded that the stimulus intensity at the site of action is presumed to be related to the dose rate. The minimum dose rate was determined, and again it was found to vary with the species

tested. When they made a moth ERG, the threshold of the ERG was the same as that for initiation of wingbeat, a fact which suggests that induction of flight activity in this case is a behavioral consequence of visual stimulation. (39)

If the photoreceptors were the sole locus of reception to x-rays, the animal should respond as though the stimulus was light in either a phototaxic or photophobic manner. As previously stated, other parts of the organism are radio-sensitive and show an immediate response to irradiation. Thus the response may result from the stimulation of a complex of receptors, yet one type of receptor may be the most sensitive. If the behavioral response is quite noticeable, one can test the photoreceptors for this property of sensitivity by simply excising them and observing any change in the response. If the animal no longer exhibits the response, or if the response is attenuated, one can conclude that the photoreceptors might have a major role in radiation perception.

Method and Materials

Animals: The fiddler crab, Uca pupillator, was selected for these studies for several reasons. First and most important, this animal has its photoreceptors located at the end of protruding eyestalks thus making excision a relatively simple procedure. Secondly, the animal is in a proper size and weight range to fit within a uniform radiation field. Finally, the crab normally exhibits a measurable locomotor activity, variations of which can be used as an experimental parameter.

Radiation: 150 KVp x-rays were produced by means of a Campbell x-ray therapy unit. Dose rates could be varied by raising or lowering the test chamber. The radiation characteristics for all the experiments were 150 KVp at 8 milliamps, filtered through 5mm. of aluminum inherent in the tube itself.

Dosimetry: Doses and dose rates were determined by means of a Victoreen condensor r meter (Model 70) and a 250 r thimble chamber probe. For each experiment three determinations of dose were taken, and the values were averaged to give the final figure. Dose rates varied from a maximum of 240 r/min. to a minimum of 15 r/min.

Recording: Graphic recording of gross locomotor activity of the fiddler crab in the test chamber was accomplished by means of a Sanborn Recorder utilizing a Sanborn strain gauge amplifier. In this experiment SR-4 paper bonded strain

gauges were attached to both sides of a .25mm. thick piece of aluminum. A bridge connected the two sides in order to sense the slight difference in resistance caused by the minute bending of the aluminum. One end of the aluminum was attached to a solid base whereas the other end supported the test chamber, a small plastic dish. A change in tension of the aluminum strip due to the movements of the animal was transformed via a corresponding change in resistance of the strain gauge into an electrical impulse recorded on the polygraph.

Analysis of Data: An indication of the application and removal of stimulation was made on the polygram. A marked amplification or attenuation of the recording indicated a noticeable increase or decrease in locomotor activity, respectively.

Subjective observations of behavior: The x-ray unit was equipped with a lead shielded window through which variations in activity could be observed. The animal was illuminated with a red light to make it visible to the observer.

Experimental design: Before each experiment the animals were placed in the dark for ten minutes. The first experiment was concerned with determining dose rate dependency of the off response. The total dose was held constant at 16.5r, and three dose rates, 15r/min, 140r/min, and 240r/min, were applied to the animals. After first ligating the eyestalks at the movable joint near the proximal end of the stalk,

they were then removed from ten crabs. After a day to allow for healing, these animals were subjected to a dose rate of 240r/min. for four seconds. A light control was run by recording the off response to a light stimulus of ten normal and ten blinded crabs in the same test chamber.

Results

A. Gross behavioral responses of Uca pugilator to whole body x-irradiation and light.

Both subjective observations and electronic recording techniques showed similar results. All the results were obtained with the test chamber illuminated by dim red light. If the dose rate were sufficiently high, above 140 r/min. the animals would exhibit a characteristic behavioral response when the radiation stimulus was turned off. This response consisted of a transient (up to two seconds) cessation of locomotor activity. The response was observed in 40 of 60 trials at 240 r/min. In some experimental runs, 10 of 10 trials exhibited this response. The response was seen only after the cessation of the radiation stimulus, and the animal exhibited no obvious behavioral change either at the onset or during irradiation. A typical electronic recording is shown in Fig. 1. There is an almost instantaneous response as can be seen by the diminished amplitude of the recording, following the indication of removal of stimulus. Unfortunately, the recording system, because of its inherent properties, was not as sensitive an indicator of this type of response as a subjective observation. In order to minimize personal error, on several occasions two people simultaneously, but independently, observed the results with good correlation of observations. Responses were recorded as positive (definite stop), questionable, or negative (no response). At low

dose rates, 15 r/min, 49 of 60 trials were negative, and 15 were questionable. Only one off response in sixty was noticed at this dose rate.

Another 100 trials were performed in which light served as the stimulus source. The recording chamber and recording procedure were identical to those used in the studies on x-irradiation. Animals, under the constant illumination of dim red light, were exposed to light from a microscope lamp. When the light was turned off, they showed the characteristic cessation of movement. To eliminate the possibility that the switch noise might be the clue, the light was turned off by pulling the plug. Ninety-five of one hundred trials gave a positive off response. It was also noted that when light and x-rays were applied concurrently, the animal would not respond obviously to the x-rays.

B. The effects of whole body x-irradiation and light on eyestalkless Uca pugilator.

The recording chamber and recording procedure were again identical to those in the previous set of experiments. Forty trials were performed at a dose rate of 240 r/min, and a total dose of 16.5 r. There was not one positive response in the forty trials attempted.

When, under the same conditions, light was used as a stimulus, not one trial in forty showed the characteristic response to cessation of stimulus.

C. The effects of dose rate on the response of Uca pugilator to whole body irradiation.

TABLE I

<u># of trials</u>	<u>Dose rate</u>	<u>Total dose</u>	<u>Off response</u>		
			+	?	o
60	15r/min	16.5r	1	49	15
60	140r/min	16.5r	15	37	8
60	240r/min	16.5r	40	11	7

Discussion

There are three probable ways that x-rays might evoke the characteristic response noted in this experiment. First, there could be an indirect activation of the nervous system by the release of bioactive substances such as endocrine secretions or biogenic amines. Secondly, there might be a direct activation of the optic nerve or some other neural structures. Finally, there might be a direct activation of the photoreceptors.

The first possibility, that of an indirect activation by the radiation induced release of endocrine substances, can not be discounted entirely. However, a number of bits of indirect evidence would suggest that this possibility is unlikely. First of all, a response to endocrine stimulation would be slower than the instantaneous response witnessed in the fiddler crab. Previous studies have shown that the release of neurosecretory substances by irradiation usually exhibits a much longer latent period and requires a much higher total dose than that applied in these experiments. (19, 31, 32, 36) Secondly, most endocrine structures remained intact in the animal when the eyestalks were removed, and since the response was no longer apparent upon excision of the eyestalks, one can eliminate other neuroendocrine structures as possible loci, instrumental in causing the response. However, one can not entirely discount the possibility that the sinus gland and its related structures,

located within the eyestalks, might be involved in the observed response. Nevertheless, it seems unlikely that the sinus gland is involved since there does not seem to be any reported behavioral off response, such as that observed in this experiment, which has been attributed to an endocrine system.

The second possible locus for evoking the observed response are neural structures, such as the optic nerve, etc. It also seems to be unlikely that these are the target structures. If the nerves were stimulated directly, one should be able to evoke a response in the eyeless animals with the same dose and dose rate utilized in this study. This did not prove to be the case. It is also known from previous investigations by a large number of workers that the threshold dose of irradiation to neurons, necessary to produce any change in excitability, is thousands of roentgens, (17, 40) The response in this experiment was evoked with total doses of as little as 16.5r.

The results of this experiment strongly suggest that the photoreceptors are the primary locus of x-ray reception in the fiddler crab. The speed of the response strongly implies a photoreceptor activation. The similarity of the off response to light and the off response to x-rays was apparent. In each case there was a distinct hesitation upon removal of the stimulus. The many investigations, previously reviewed in the Introduction, tend to support the contention that the

photoreceptor, in all probability, is the x-ray sensitive structure. Finally, and perhaps most important, when the photoreceptors were excised, the characteristic off response to both light and x-rays was abolished.

The mechanism by which x-rays evoke an activation of the photoreceptors was not elucidated by these experiments. However, one can speculate that there could be a direct activation of a photosensitive molecule by means of the radiation induced excitation of molecules. The molecule in this excited state would be able to undergo either an already known series of reactions or a different series in which its newly acquired energy could be utilized to activate a photosensitive molecule such as rhodopsin.

Baylor and Smith (6), as well as Lipetz (27), suggested that indirect activation of photosensitive molecules by the production of fluorescence was unlikely. However, this source of activation can not be definitely eliminated as a possibility.

In a number of previous studies, reviewed earlier in this paper, it has been noted that many behavioral responses are dose-rate rather than total dose dependent. The results of this study also indicate a response dependent on dose-rate. The question that occurs is why should the rate of energy application rather than the total energy applied be a critical factor in a number of systems involving receptor cells.

One attractive hypothesis involves a system with a fixed repair rate, for example, a sodium pump or the resynthesis of rhodopsin, and also with a given response threshold. If this condition exists, as it does in neurons and receptors, then the rate of stimulus application becomes important. This stimulus characteristic is found in biological systems where temporal summation occurs. If the rate of energy absorption exceeds the rate of repair, then the threshold can be attained. If, on the other hand, the rate of repair either equals or is greater than the rate of energy absorption, no response is evoked. The strong dose-rate dependency, observed in this study, might be explained by the above hypothesis. Although other possible mechanisms for dose-rate dependency exist, no critical work has been done to elucidate this phenomena which has only recently come to our attention.

Conclusion

There are three final conclusions to be derived from the results of this experiment. First, one can state that the fiddler crab exhibits an instantaneous behavioral response to the removal of the x-ray stimulus. Secondly, the response demonstrated by this animal is dependent on the dose-rate. Finally, although this conclusion can not be stated unequivocally, one can strongly suggest that the photoreceptors of this animal are the primary locus of x-ray perception.

Bibliography

1. Andrews, H. L., and Cameron, L. M., "Radiation Avoidance in the Mouse." Proc. Soc. Exp. Biol. & Med., 103, 565-567, (1960).
2. Axenfeld, D., "Behavioral Responses of Animals to X-irradiation." Naturw. Rundschau, 11, 607-612, (1896) cite Krebs.
3. Bachofer, C. F., and Wittry, S. E., "Interactions of X-rays and Light in the Production of the ERG." Exptl. Eye Res., 2, 141-147, (1963).
4. Bachofer, C. F., and Wittry, S. E., "Off Response of ERG Induced by X-ray Stimulation." Vision Res., 3, 51-59, (1963).
5. Baldwin, W. F., Sutherland, J. B., and Habowsky, J. E. J., "Effects of X-rays on Electrical Activity in the Eye of the Cockroach, Blaberus giganteus." Nature, 199, 616, (1963).
6. Baylor, E. R., and Smith, F. E., "Animal Perception of X-rays." Radiation Research 8, 466-474, (1958).
7. Born, W., "Release of Reflexes in Snails by x and d-rays." Strahlentherapie, 112, 634-636, (1960).
8. Bornschein, H., Pape, R., and Zakowsky, J., Naturwissenschaften, 40, 251, (1953) cite Lipetz.
9. Brown, W. L., Overall, J. E., Logie, L. C., and Wicker, J. E., "Lever Pressing Behavior of Albino Rats during Prolonged Exposure to X-radiation." USAF School Av.

Med. Report., 60-66, (1959).

10. Castanera, T. J., Jones, D. C., and Kimeldorf, D. J.,
"The Effect of X-irradiation of the Diffuse Activity
Performance of Rats, Guinea pigs, & Hamsters."
Brit. Journ. of Radiology, 32, #378, 386-389, (1959).
11. Chaluppecky, H., Zbl. Prakt. Augenheilk., 21, 234, 267,
(1897) cite Lipetz.
12. Dawson, W. D., and Smith, J. C., "Low Level Irradiation
and Threshold Shift in the Visual Receptor."
Science, 129, 1670-1671, (1959).
13. Garcia, J., Buchwald, N. A., Feder, B. H., and Koelling,
R. A., "Immediate Detection of X-rays by the Rat."
Nature, 196, 1014, (1962).
14. Garcia, J., Kimeldorf, D. J., and Hunt, E. L., "The Use
of Ionizing Radiation as a Motivating Stimulus."
USNRDL-TR-411, (1960).
15. Garcia, J., Kimeldorf, D. J., and Koelling, R. A.,
"Conditioned Aversion to Saccharin Resulting from
Exposure to Radiation." Science, 122, 157-158,
(1955).
16. Goldhaber, G., and Feldman-Muhsam, B., "Immediate Effects
of X-rays on the Movements of Larvae & Pupae of
Mosquitoes." Nature, 153, 528, (1944).
17. Haley, T. J., and Snider, R. S., Response of the Nervous
System to Ionizing Radiation. Academic Press, New
York and London, 533-596, (1962).

18. Himstedt, F., and Nagel, W. H., "Action Current in X-irradiated Frog Eyes." *Phys. Zs.*, 2, 362-368, (1901) cite Krebs.
19. Hopsu, V. K., Talanti, S., and Voutilainen, A., "Effects of Whole-body Irradiation on the Neurosecretory Material of the Rat Hypophysis." *Acta. Radiol.*, 54, 220-224, (1960).
20. Hug, O., "Reflex-like Responses of Lower Animals and Mammalian Organs to Ionizing Radiation." *Immediate and Low Level Effects of Ionizing Radiations*, Conference held in Venice, June, 1959.
21. Hug, O., *Intern. Journ. Radiation Biology Spec. Supply*, 2, 217, (1960).
22. Hunt, E. L., and Kimeldorf, D. J., "Evidence for Direct Stimulation of the Mammalian Nervous System with Ionizing Radiation." *Science*, 137, 857 (1962).
23. Kektcheew, K., "Disturbance in Retinal Function by Weak Irradiations." *Problemy Fiziol. Opt.* 1, 77-82 (1941) cite Krebs.
24. Kimeldorf, D. J., Garcia, J., and Rubadeau, D. O., "A Demonstration of Radiation Induced Behavior in Cats, Mice, and Rats." *Rad. Research*, 9, #1, 139 (1958).
25. Krebs, A. T., "Immediate Reaction to X-irradiation of the Red Eared Turtle, Pseudemys scripta elegans." U.S. Army Med. Research Lab Report #445, (1960).

26. Levy, C. K. (personal communication).
27. Lipetz, L. E., "The X-ray and Radium Phosphenes." Brit. Journ. Ophthal., 39, 577-597 (1955).
28. Lipetz, L. E., "The Electrophysiology of the X-ray Phosphene." Rad. Res., 2, 306-329 (1955).
29. Lipetz, L. E., "The Effects of Low Doses of High Energy Radiation on Visual Functions." Reprint from Immed. and Low Level Effects of Ionizing Radiation Conference, (1959).
30. Logie, L. C., Brown, W. L., Pizzuto, J. S., and Overall, T. E., "Some Evidence Suggesting the Perception of Moderate Intensity Radiation among Albino Rats." USAF School Av. Med. Report 60-65 (1959).
31. Maiorova, V. F., "Effect of X-irradiation on the Neurosecretory Systems." Fed. Proc., Transl. Suppl., 22:T1187-90, (1963).
32. Musailyan, S. S., and Sytinski, I. A., "The Effect of Total X-ray Irradiation on the Level of x-amino-butyric Acid in the Brain." Doklady Akad. Nauk S.S.S.R., 139, 994-995, (1961).
33. Olivard, T., and Hungate, F. P., "Tests for the Ability to Sense Ionizing Radiation." Quarterly Progress Report HW-57908 (1958) cite Krebs.
34. Overall, J. E., Brown, W. L., and Logie, L. C., Brit. Journ. Radiol. 32, 411 (1959).

35. Overall, J. E., Logie, L. C., and Brown, W. L., "Changes in the Shuttle-box Behavior in Albino Rats in Response to X-irradiation at 1r/min." *Rad. Research* 11, #4, 589-599 (1959).
36. Randic, M., and Supek, Z., "Influence of high Doses of X-irradiation on 5-HT in Brain of Rats." *Intern. J. Radiation Biol.*, 4, 637-638 (1962).
37. Rugh, T. C., and Fulton, J. F., Medical Physiology and Biophysics, ed. 18, W. B. Saunders Co., Philadelphia and London, (1960).
38. Scarborough, B. B., and Addison, R. G., "Conditioning in Fish; Effects of X-irradiation." *Science*, 136, 712 (1962).
39. Smith, J. C., Kimeldorf, D. J., and Hunt, E. L., "Motor Responses of Moths to Low-intensity X-ray Exposure." *Science*, 140, 805-806, (1963).
40. Van Cleave, C. D., Irradiation and The Nervous System. Roman and Littlefield, Inc., New York, Chaps. 6, 10, 11 (1963).
41. Veninga, T. S., "The Frog's Eye ERG as an Immediate X-ray Effect." *Koninkl. Ned. Akad. Wetenschap, Proc. Ser. C.*, 64, 208-211 (1961).

Abstract

Many animals, invertebrates as well as vertebrates, have demonstrated an ability to somehow sense ionizing irradiation. This recognition is often apparent by a behavioral response which can be correlated with the x-ray stimulus in some way.

The fiddler crab, Uca pugilator, was found to exhibit a behavioral response to ionizing irradiation. When the x-ray machine was turned off, the animal would respond instantaneously by a marked hesitation in its general movement after which it would resume its normal activity. This response suggests the animal's ability to somehow be aware of irradiation. Previous work in this area suggested that the photoreceptors were the primary site of stimulation. The fiddler crab's photoreceptors, located at the ends of protruding eyestalks, are particularly easily excised. When the eyestalks were removed, the response to x-rays was no longer evident. A parallel series of experiments were done with light as the stimulus. With intact eyestalks, the animal showed the same off response, and with the eyestalks removed, the subject exhibited no such response. The possibility of a direct stimulation of nervous structures as well as that of an indirect activation by the x-ray evoked release of bioactive substances is discussed. After considering the sinus gland, which was also removed along with the photoreceptors, as a possible site of x-ray reception, a strong implication that the photoreceptors are the primary locus of x-ray sensitivity in the fiddler

1

crab was stated. The marked dose rate dependency of the animal's response to x-rays was noted, and a possible explanation was suggested.

2