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Radiation damage to the sixth abdominal ganglion of the crayfish, *Procambarus clarkii*

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RADIATION DAMAGE TO THE SIXTH
ABDOMINAL GANGLION OF THE
CRAYFISH, *PROCAMBARUS CLARKII*

A Thesis

Presented to

The Faculty of the Department of Biological Sciences
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by

Albert Vitaly Bystritskii

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ABSTRACT

RADIATION DAMAGE TO THE SIXTH ABDOMINAL GANGLION OF THE CRAYFISH, *PROCAMBARUS CLARKII*

by Albert V. Bystritskii

The early delayed effects of exposure of the crayfish, *Procambarus clarkii*, nerve cord to ionizing radiation were examined in relation to the response of the sixth abdominal ganglion to light and overall spontaneous activity of the nerve cord. A goal of this project was to develop a model for studying delayed radiation damage to neurons in the nervous system. Crayfish were exposed to gamma-radiation with dose in the range of 106-159 Gy. The irradiated group (N=17) displayed significant differences ($p < 0.05$) from the control group (N=15) in 1) spontaneous bioelectric activity during dark adaptation, 2) response time to light stimulation, 3) frequency of spike potentials in response to a light, and 4) time required for dark adaptation. The radiation damage to the crayfish nervous system manifested as an overall irritability which could be explained as an increase in the membrane permeability after irradiation.

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This thesis is dedicated to my wife, Anna, who motivated me in the difficult times, and to our future.

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INTRODUCTION

Studies of ionizing radiation and its effect on the biological organisms began at the same time as the discovery of radioactivity (1). The effects of radiation on different tissues and organs have been described (2, 3). The nervous system was usually considered to be relatively radioresistant. However, studies made in the 1960's showed that ionizing radiation can affect the nervous system over a broad range of doses and dose rates (4).

The initiation of biological effects is thought to result from the energy deposited directly within specific sensitive target molecules or transmitted indirectly to such molecules through the intervention of chemical reaction products formed by the interaction of the radiation with the aqueous environment. The cellular reactions to molecular alterations range from transient disturbances in metabolic process to death of the cell (4).

Ionizing radiation has been shown to have a number of effects on the nervous system and biological membranes. Increased irritability and excitability of sensory receptors after radiation exposure with different doses, especially photoreceptors in a variety of organisms, were described by Levy in 1967 (5). Radiation activation of pacemaker cells in invertebrates was described by Bailey and Noell (6) as an increased frequency of spontaneous discharge. Ionizing radiation has been shown to change membrane permeability to Na^+ and K^+ ions in invertebrates (7), and to release membrane-bound calcium from red blood cells in humans (8). Gamma-radiation has also been shown to affect synaptic transmission by releasing calcium ions from presynaptic membranes (9). All these studies noted that the observed effects were more dependent on dose rate than on the total dose of the exposure.

We have used the crayfish as a model to investigate the effects of ionizing radiation on the nervous system. Arthropods, specifically Crustaceans, have been studied by biologists since the Renaissance. The crayfish in particular has played a prominent role in the development of modern neurobiology (10). The crayfish was chosen as a model to study bioelectric phenomena in neurons because of its ready availability, ease of maintenance, and the relative viability of the nervous system after exposure and or removal. Individual neurons in the nerve cord have now been identified and studied, and neuronal maps have been drawn (11, 12).

The crayfish possess an unusual, perhaps unique, caudal photoreceptor (CPR), located in the sixth abdominal ganglion, first described by Prosser in 1933 (10). Kennedy (13) determined that there are only two photosensitive neurons in the sixth ganglion, and that they are complex, secondary interneurons, that also receive inputs from tactile receptors in the tail. Studies of leg movements in the crayfish by Welsh (14) have shown that significantly greater leg activity resulted when the caudal ganglion was exposed to light than with light exposure to the eyes. Investigators have examined such properties of the CPR as its light sensitivity, its wavelength action spectrum, and the effect of light exposure on behavioral reactions. The CPR can act as a substitute for the eyes as an input in regulating the diurnal activity cycle, and it is involved in initiating the abdominal flexion escape reaction (15, 16, 17). The light action spectrum of the CPR was found to be similar to that of the eye photoreceptor pigment, rhodopsin, with the sensitivity peak at 500 nm (18, 19). The CPR also possesses pacemaker activity, generating spontaneous spike potentials even in

complete darkness and this spontaneous spiking increases after light stimulation (13).

The immediate effects of ionizing radiation on the CPR of the crayfish were described by Rodriguez and Kimeldorf (20), who showed: (a) ionizing radiation (x-ray) is detected by the CPR in a similar manner to light; (b) radiation exposure initiated an avoidance behavioral response; and (c) general neural excitation. The authors also showed that the bioelectric response of the CPR was characterized by a gradual increase in frequency of spikes shortly after exposure began and an after-discharge following termination of the exposure. The magnitude of the discharge or spike frequency was intensity or dose rate dependent. These observations were made on both low and high amplitude spikes, indicating that more than one group of neurons were involved in the recorded response. The authors hypothesized that these changes were due to the release of membrane-bound calcium at the synapse by ionizing radiation, these synapses involved neurons other than the CPR.

The studies described here examine the early delayed response (from one to two days after irradiation) of the nerve cord to ^{137}Cs gamma-radiation. Our studies follow the studies conducted by Rodriguez and Kimeldorf (20), in which radiation served as a stimulus and the observed phenomena were immediate responses. It has been shown by Darden in 1960 (21), Gaffey in 1962 (22), and Levy in 1967 (5), that ionizing radiation increases the membrane permeability to ions, which, possibly, will lower the threshold of depolarization. The increase in membrane permeability could result from direct damage to the lipid bilayer, changes in ion channel properties, and direct damage to the sodium-potassium pump (23). Another possible result of the membrane

permeability changes could be increased calcium leakage from synaptic membranes of neurons, and this may increase spontaneous discharges by releasing neurotransmitters (8, 24). Brinkman (25) described cases where irradiation of the head in mammals produced a release of serotonin, histamine and epinephrine. We hypothesize that the early delayed effects of ionizing radiation will increase the spontaneous bioelectric activity of the crayfish abdominal nerve cord, and also alter the response of the sixth abdominal ganglion to light stimulation.

Another goal of the present research was to develop a model for studying delayed radiation damage to neurons in the nervous system *in vivo*. In order to do this the parameters of the model need to be characterized and defined. Once the model is characterized, then pharmacological and ion concentration studies can be done to test the system with respect to membrane permeability and synaptic transmission.

The early delayed effects of exposure of the crayfish nerve cord to ionizing radiation were examined in relation to the response of the sixth abdominal ganglion to light stimulation. The extent of the radiation damage was determined in control and irradiated crayfish by measurements of 1) the spontaneous bioelectric activity in the nerve cord during dark adaptation, 2) the response time to light stimulation (response delay), 3) the frequency of spike potentials in response to a light stimulation, and 4) the time required for dark adaptation after prolonged exposure to light.

MATERIALS AND METHODS

CRAYFISH

Male and female crayfish, *Procambarus clarkii* (Girard) 7-12 cm long, were obtained from Niles Biological company, Sacramento, CA. The crayfish were maintained at 5°C in fresh water. Crayfish were anesthetized on ice for 5-6 minutes to achieve anesthesia prior to dissection. The animals were immobilized on a specially constructed Styrofoam block and the exoskeleton on the ventral side of the abdomen was carefully removed to expose the ventral nerve cord and the associated ganglia. The nerve cord was cut between first and second abdominal ganglia in order to isolate and record only afferent electrical activity generated from the more posterior ganglia, mainly the sixth ganglion. The crayfish with the exposed nerve cord was placed in chilled Van Harreveld's saline solution (NaCl 205 mM; KCl 5.4 mM; MgCl₂ 2.6; CaCl₂ 13.5 mM; NaHCO₃ 2.3 mM; pH 7.5-7.6) (26).

ELECTROPHYSIOLOGY

Bioelectrical activity of the nerve cord was detected with suction electrodes (24). The electrodes were made from a Pasteur pipette and Ag-AgCl wire attached to Tygon tubing and a 5 cc syringe. The electrode was mounted on a micromanipulator and the tip placed at the cut end of the nerve cord adjacent to the second abdominal ganglion. Slight suction was generated with the syringe and the end of the nerve cord was drawn into the saline filled electrode. The afferent electrical activity (action potentials) that originated from the more posterior portion of the nerve cord was recorded in this manner during the period of dark adaptation and after light stimulation. The crayfish was

allowed to rest and dark adapt for at least 15 minutes. During the period of dark adaptation the baseline spontaneous activity of the nerve cord was recorded. The activity of the light-sensitive sixth abdominal ganglion was observed and recorded after exposure to light from a high intensity surgical lamp, placed 50 cm from the crayfish. The activity of the stimulated nerve cord was followed for at least 10 minutes during light stimulation, until a plateau phase was reached; then the light was switched off. The plateau phase was considered to be achieved when the spike potential frequency remained constant over at least 3 min. Thus the spike activity of the nerve cord was recorded before, during, and after light stimulation. Response time was determined by measuring time between the onset of light stimulation and the increase in spike potential frequency by at least 50%.

The parameters that were measured and obtained from these data were 1) spike potential frequencies, 2) response times after onset of light stimulation, 3) dark adaptation time after the light stimulation was switched off, and 4) frequency of large versus small spike potentials.

The suction electrode was connected via a head stage to a preamplifier (Mentor N-950). The electrical activity was further amplified on an oscilloscope (Tektronix 5810N). The bioelectrical activity was recorded on an PC AT-486 computer (American Megatrends Inc.). A software package, Computerscope-EGAA 3.40.00, by RC Electronics Inc. was used for recording and storing data. The program created an electronic chart recorder display on the computer monitor and stored the data over a period of 110 seconds. Single screen displays were printed out and the number of spikes per second were counted manually. The spikes higher than 7 mm were considered to be high amplitude.

Spikes lower than 7 mm, but above background noise were classified as low amplitude spikes.

IRRADIATION

A ^{137}Cs Mark 1 irradiator (J.L. Shepherd & Ass., model 25) located in the Nuclear Science Facility at SJSU, was used as a source of gamma-radiation. Radiation dosimetry was done by exposing a standard Fricke solution (0.001 M $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, 0.001 M NaCl, 0.8 M H_2SO_4) at approximately 5 cm from the source. The dose was determined by measuring ferric yield (absorbance at 305 nm) and using a G value of 15.3. The dose rate was found to be 10.59 ± 0.17 Gray/min (Gy/min) ($1 \text{ Gy} = 100 \text{ rad} = 1 \text{ J/kg}$). This result was confirmed by measurements with a Victoreen ion chamber and R meter.

For irradiation, crayfish were immobilized on the styrofoam mounting block and placed in the irradiator chamber 5 cm from the source. The time of exposure varied between 10 and 15 minutes, which corresponds to a dose range of 106-159 Gy (high dose). All animals were exposed to this high dose of radiation.

After irradiation, the crayfish were placed in a freshwater tank for at least 24 hours and maintained at 5°C before recording electrical activity.

STATISTICS

Standard statistical analyses of the response parameters were made and the Student's t-test was used to determine significance. A one tailed test was made at $p < 0.05$ (25).

RESULTS

The dose was determined from the product of the time of exposure and the dose rate that was measured with the Fricke dosimetry. There was no significant effect on spike potential frequency after exposure to doses less than 100 Gy (data not shown). Figure 1 summarizes the response of the sixth abdominal ganglion and the abdominal nerve cord in control and irradiated crayfish. During a period of dark adaptation, before light stimulation, the spontaneous spike potential frequency was significantly higher ($p < 0.001$) in the irradiated group than in controls. The average frequency of afferent spike potentials in pulses per second (pps) was 34 ± 14 for the controls ($n=15$) and 63 ± 12 for the irradiated group ($n=17$). The frequency of spike activity was also significantly ($p < 0.001$) higher in the irradiated group when exposed to light than in the control group. As can be seen in figure 1 this was the case for the initial response to light (within 10 sec) and after the plateau phase was achieved. For the control group the frequency of spike activity was 90 ± 26 pps and 86 ± 18 pps for the initial and plateau response respectively. For the irradiated group it was 124 ± 22 pps and 117 ± 16 pps. There was a slight drop in frequency in both groups after prolonged light stimulation (plateau), compared to the initial response (see data in Table I).

Figure 2 displays frequencies of spike potentials of different amplitudes during the initial response to light, and during dark adaptation for both control and irradiated groups. The average frequency of high amplitude spikes was greater in the irradiated group than in the control group ($p < 0.001$) for

Figure 1. Spike potential frequencies of control, and irradiated groups of crayfish. Recordings were made for both groups during dark adaptation, immediately after light exposure (initial response within 5 sec), and during the plateau phase (at least 30 sec after the onset of the light stimulus). The error bars indicate standard deviation from mean values. All differences in the means are significant to the 0.001 level (Student's t-test). N=15 for controls, N=17 for irradiated group.

TABLE I
Spike potential frequencies of control (N=15), and irradiated
(N=17) groups of crayfish.

Group	Mean	Standard deviation
Control dark adapted	34.1	13.86
Irradiated dark adapted	62.85	12.35
Control light response	89.65	25.69
Irradiated light response	124.09	22.21
Control plateau of light response	85.95	17.92
Irradiated plateau of light response	117.31	15.59

dark adapted crayfish as well as for the initial response to light. The average frequency of high amplitude spikes for the control group was 14 ± 7 pps for dark adapted crayfish, and 46 ± 15 pps after light stimulation. The average frequency of high amplitude spikes for the irradiated group was 27 ± 10 pps and 73 ± 21 pps during dark adaptation and the initial light response, respectively. The average frequency of low amplitude spike potentials was also found to be higher for the irradiated group than for the control group ($p < 0.001$). The average frequency of low amplitude spikes for controls was 21 ± 8 pps for dark adapted crayfish and 43 ± 19 pps for the initial response to light. For irradiated crayfish the frequency of low amplitude spikes was found to be 37 ± 7 pps for dark adapted, and 50 ± 10 pps for the initial response to light (see data in Table II).

Figure 3 shows the average response time to light stimulation. It was found to be significantly longer for the irradiated group, 2.09 ± 0.38 sec, than for the control group, 1.22 ± 0.23 sec ($p < 0.001$).

Figure 4 displays the dark adaptation process for both control and irradiated groups over time. The crayfish were assumed to be fully dark adapted when the frequency of spike potentials occurred at the same rate as before light stimulation. The spike potential frequency decreases with time during the dark adaptation period. The time required for dark adaptation of irradiated crayfish (about 10 min.) is significantly longer than for controls (about 5 min.), ($p < 0.001$).

Figure 5 shows a typical display of the spontaneous spike activity for control dark adapted crayfish. As can be seen, the time distribution of spike potentials is relatively uniform. This spontaneous activity consists of small

Figure 2. High and Low Amplitude spike potential frequencies of control, and irradiated groups of crayfish. (High amplitude spikes > 7mm, low amplitude spikes < 7 mm). Error bars indicate standard deviation from mean values. All differences in the means are significant to the 0.001 (Student's t-test). N=15 for controls, N=17 for irradiated group.

TABLE II
High and Low Amplitude spike potential frequencies of control
(N=15), and irradiated (N=17) groups of crayfish.

Group	Mean	Standard deviation
Control, dark adapted, High Amplitude	13.58	7.01
Irradiated, dark adapted, High Amplitude	26.91	9.58
Control, light response, High Amplitude	46.42	14.56
Irradiated, light response, High Amplitude	73.34	21.48
Control, dark adapted, Low Amplitude	20.45	8.11
Irradiated, dark adapted, Low Amplitude	36.91	7.00
Control, light response, Low Amplitude	43.23	19.32
Irradiated, light response, Low Amplitude	50.80	10.04

Figure 3. Average delay of the initial response to the light stimulation in control, and irradiated groups of crayfish. Error bars indicate standard deviation from mean values. All differences in the means are significant to the 0.001 level (Student's t-test). N=15 for controls, N=17 for irradiated group.

Figure 4. Time course of dark adaptation process in control (N=15, black circles), and irradiated (N=17, white circles) groups of crayfish. Light was turned off at 0 sec. The frequency was measured every 10 sec. Error bars indicate standard deviation from mean values. All differences in the means are significant to the 0.001 level (Student's t-test).

Figure 5. Typical recording of dark adapted crayfish (control group). Note the low frequency of high amplitude spikes and uniform distribution of spike potentials.

potentials interspersed with large potentials characteristic of the neurons in the sixth ganglion.

Figure 6 displays the typical initial response to light by the nerve cord of the control crayfish. Despite the increase in spike potential frequency, the time distribution of spike potentials, both large and small, was also relatively uniform.

Figure 7 presents the recording from the nerve cord of irradiated dark adapted crayfish. In this figure the spike potentials are distributed unevenly and appear to occur in bursts. The large size of the spike potentials indicates that they originate from the sixth ganglion.

Figure 8 shows the characteristic picture of initial response to light stimulation of the irradiated crayfish. Here it is noticed that, together with an increase in frequency, the character of spike distribution is also different than is shown in figure 8. It is best described as "burst" activity with groups of high amplitude spikes with intermittent relatively silent periods. Each burst consists of a high frequency of both small and large spikes.

Figure 6. Typical recording of initial light response (control group). Spike potential frequency increased, compared to dark adapted; the distribution pattern of the spike potentials stays uniform.

Figure 7. Dark adapted crayfish (irradiated group). Note the spike potential distribution pattern. Quiet periods are followed by bursts of spontaneous activity. The frequency of high amplitude spikes is greater than in the control group.

Figure 8. Initial response to light (irradiated group). Note the increase in spike potential frequency, compared to dark adapted crayfish (fig. 7). the distribution pattern is almost identical to fig. 7, with high amplitude spike potentials grouped in bursts.

DISCUSSION

The irradiated group of crayfish showed significant differences from the control group in the electrophysiological response of the CPR during dark adaptation and during light stimulation.

The average frequency of spontaneous afferent spike potentials during dark adaptation and during light stimulation was higher in the irradiated group than in the control group. This phenomenon might be explained as a general increase in irritability of neurons, particularly of the CPR. These results correlate with results of Darden, and Gaffey, who found that ionizing radiation in high doses produce an increase in membrane permeability to sodium and potassium ions (21, 22). The increased membrane permeability to sodium ions would bring the membrane potentials close to the firing threshold, thus increasing the excitability of neurons. The possible mechanisms of increased membrane permeability are direct damage to the lipid bilayer, which can alter the properties of the ion channels, or direct damage to the ion channels. Partial damage to the sodium-potassium pump (23) could result in a partial depolarization of the resting membrane potential, thus bringing the neuron close to the firing threshold, which could increase the spike potential frequency. Permeability changes could also result in an increased leakage of calcium into the presynaptic membranes of neurons. The increase in internal calcium may increase spontaneous discharges, caused by the release of neurotransmitters, such as glutamate, acetylcholine, and serotonin, which are excitatory neurotransmitters in the crayfish nervous system (24, 25, 29).

The average initial response time to light stimulation is significantly longer for the irradiated group than for the controls. There is more than one

possible explanation for these results. The mechanism of the CPR response to light stimulation is similar to those of the eyes (20). This process is mediated by the visual pigment rhodopsin. The initial event is the absorption of a photon of suitable energy by a visual pigment molecule. This then causes an intramolecular energy transfer that breaks chemical bonds and triggers a sequence of events ending with a rapid change in electric potential, a bioelectric signal. These reactions take certain amount of time, thus causing the delay of response to stimulation. Radiation damage of the visual pigment system may cause the increase in delay of response to light stimulation. Another possible explanation of increased response delay could be the activation of inhibitory neurons. Kennedy, Calabrese, and Wine (30) described the presynaptic inhibition of transmission between tactile sensory neurons and interneurons in the caudal ganglion of the crayfish. These synapses are axo-axonal at a point near the presynaptic membrane, and protect the crayfish from the sensory input overload (Figure 9). The authors showed that inhibition is correlated with a hyperpolarization of the presynaptic membrane, thus increasing the threshold for firing. The hyperpolarization is accompanied by a postsynaptic membrane conductance increase, which can possibly be explained by efflux of potassium ions, or influx of chloride ions. The hyperpolarization process is mediated by inhibitory interneurons that, when excited, can alter the activity of the neurons which compose the CPR (13). The inhibitory neurons could become excited as a direct effect of ionizing radiation, or become excited by increased activity of CPR (Figure 9). This is a possible mechanism for the increase of response time. The significant increase in response delay to light in the irradiated group, compared to that of the control

Figure 9. Simplified circuit diagram of connections between the CPR, the inhibitory neurons, and the following ganglia. Abbreviations: VI, sixth ganglion; V, fifth ganglion; IV, fourth ganglion; I.I., inhibitory interneuron; A, CPR axon; IA, inhibitory neuron axon. Only one hemiganglion is shown; all elements are bilaterally symmetrical.

group leads us to believe that more than one mechanism is involved. The most likely explanations of increase in the response delay would be the combination of theories about the visual pigment system damage by radiation, and increased activity of inhibitory neurons.

The time required for dark adaptation was also significantly longer for the irradiated crayfish compared to the controls. This finding also can be explained by increased irritability and hyperactivity of CPR as a general effect of gamma-radiation which causes the increase in membrane permeability to sodium ions (5). That would bring the CPR neurons closer to the firing threshold, thus making them more excitable.

The different character of the recordings for the control and the irradiated groups, such as the pattern of spike potential frequency distribution, can also be explained by increased irritability and excitability of CPR. This can be explained by activation of the CPR pacemaker cells by ionizing radiation (6). The membranes of pacemaker cells are naturally more leaky to ions, especially to sodium ions, which enter the cells and eventually bring them closer to the threshold of depolarization, thus allowing them to fire repeatedly (31). This is the mechanism that occurs in pacemaker cells in the heart. The radiation damage to the membrane may increase its permeability to sodium ions, bringing it close to the discharge threshold, and thus causing more frequent firing (7).

To explain the differences in frequency of different amplitude spikes, it would be necessary to conduct further research to characterize and topographically localize neurons that generate high and low amplitude spike potentials. More precise techniques, such as patch-clamping of individual

neurons could help to answer this question. Because of a more significant increase in the high amplitude spike frequency in response to light, in comparison with low amplitude spike frequency in both controls and irradiated crayfish we suggest that the CPR is the point of origin of high amplitude spike potentials. Increases in low amplitude spike frequency probably signify damage to neurons other than those in the sixth ganglion. Another possible explanation can be made with regard to the pacemaker activity. If the CPR is a pacemaker it may govern the activity of other neurons with which it synapses. The activation of the pacemaker would cause the increase in the frequency of discharge of other neurons. Our results correlate with studies, done by Rodriguez and Kimeldorf (20), that used much smaller doses of radiation, and that also detected the increase in spike potential frequency of different amplitudes, together with a general increase of crayfish locomotive activity. Since the dose range in Rodriguez' and Kimeldorf's experiments was not high enough to cause permanent damage to the neurons, the hypothesis about a pacemaker-induced increase in neuronal activity would be, probably, the best explanation of observed results.

SUMMARY

The results of the present experiments suggest that gamma-radiation affects the caudal photoreceptor of the crayfish, located in the sixth ganglion, by making it hyperexcitable; this was demonstrated by an increase in frequency of spike potentials in dark adapted irradiated crayfish as well as an increase of spike potential frequency in response to light stimulation, compared to the control group. In addition to the observation above there was a significant increase of response delay after light stimulation in the irradiated group. This result could be explained by increasing activity of inhibitory neurons after irradiation, which blocks the synaptic transmission from the CPR by the mechanism of presynaptic inhibition. The hypotheses about radiation damage to the visual pigment system could also explain the increase in response delay.

The different character of spike potentials suggests that some neurons, other than the CPR, are affected by gamma-radiation. Another possible reason for an increase in frequency of different amplitude spike potentials could be the excitation of other neurons by CPR, which possesses pacemaker properties, and can be excited by smaller doses of ionizing radiation

These results can be used to further investigate and describe the response of neurons to radiation damage.

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