

THE EFFECTS OF WHITE NOISE AND GAMMA IRRADIATION ON
AUDITORY FREQUENCY DISCRIMINATION PERFORMANCE OF
ALBINO RATS

A Thesis
Presented to
the Faculty of Graduate Studies and Research
University of Manitoba

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

by
Arlene Patricia Wiseman
September, 1968



ACKNOWLEDGMENTS

The author wishes to acknowledge the assistance and advice of the following: Dr. M. F. Halasz, Dr. J. Pear, and Dr. A. Holloway and staff of the Manitoba Cancer Treatment and Research Foundation.

ABSTRACT

We investigated the effects of 450 rads of whole-body gamma-irradiation on a maintained auditory discrimination task, complicated by a background of white noise. Two experiments were performed on 12 Holtzman rats trained in the Skinner Box to discriminate a 1 kHz, 80 db. CS+ and a 0.7 kHz, 80 db. CS-. Lever press to CS+ was always rewarded with water, while CS- responses were never reinforced. White noise (80 db) was superimposed over one-half of each daily 50 trial session, in alternated order. Experiment I investigated the effects of radiation when subjects had been previously habituated to the noise, while Experiment II examined discrimination performance when noise and radiation were introduced together.

Results of Experiment I showed that: 1. Initial introduction of white noise had a marked suppressant effect on response to CS+ (increased latencies). 2. Habituation to white noise took place within seven days (CS+ latencies returned to baseline). 3. Resensitization to white noise was brought about by irradiation. In the absence of white noise, radiation affected the CS+ latencies only slightly. 4. By the end of the first post-irradiation week, performance again returned to control levels. In Experiment II, the initial suppressant effect of white noise on CS+ was reduced by the additional presence of radiation. In the absence of white noise, differential performance under radiation was extremely stable.

TABLE OF CONTENTS

CHAPTER		PAGE
I	THE PROBLEM, INTRODUCTION AND HISTORICAL REVIEW	1
	Statement of the Problem	1
	Introduction	4
	Historical Review	5
II	MATERIALS AND PROCEDURE	19
	Experiment I	19
	Experiment II	20
	Apparatus	21
	Subjects	22
	Deprivation Schedule	22
	General Procedure	23
	Radiation Session	25
	Behavioral Measures	26
	Specific Procedures	26
III	RESULTS AND DISCUSSION	28
	Results	28
	Discussion	47
IV	SUMMARY AND CONCLUSIONS	55
	BIBLIOGRAPHY	58
	APPENDIX A - RAW DATA FOR EXPERIMENTS I, II AND NOISE CONTROL GROUP.	69
	APPENDIX B - EXPERIMENTAL DESIGN	83

LIST OF FIGURES

FIGURE		PAGE
1	Percent Change from Baseline of Daily Mean Response Latencies Under Control Treatment	29
2	Percent Change from Baseline of Mean Response Latencies for Daily Half Sessions with and without Noise. Noise Controls	30
3	Percent Change from Baseline Mean Response Latencies for Daily Half Sessions with and without Noise when Noise was First Presented 7 Days Prior to Irradiation. Experiment I	32
4	Percent Change from Baseline Mean Response Latencies for Daily Half Sessions with and without Noise. Noise First Presented During the Irradiation Session. Experiment II	33
5	Percent Change in Daily Errors of Omission and Commission from Baseline Performance. Noise Controls	34
6	Percent Change in Daily Errors of Omission and Commission from Baseline Performance when Noise was First Presented 7 Days Prior to Irradiation ..	35
7	Percent Change in Daily Errors of Omission and Commission from Baseline Performance when Noise was First Presented During Irradiation Session ...	36
8	Standard Error of Response Latencies to CSs for Subjects Receiving Control Treatment	37
9	Standard Error of Response Latencies to CSs for Subjects Receiving Noise Only	38
10	Standard Error of Response Latencies to CSs with and without Noise when Noise was First Presented 7 Days Prior to Irradiation	39
11	Standard Error of Response Latencies to CSs with and without Noise when Noise and Irradiation were Presented During the Same Session	40

LIST OF TABLES

TABLE		PAGE
1	Levels of Significance of the Response Latencies Measures Under the Different Conditions and Times of Testing in Experiment I	45
2	Levels of Significance of the Response Latencies Measures Under the Different Conditions and Times of Testing in Experiment II.....	46

CHAPTER I

THE PROBLEM, INTRODUCTION AND HISTORICAL REVIEW

Statement of the Problem

Behavioral studies use a variety of experimental techniques which provide some insight into the modes of action of the nervous system, but results usually produce as many important questions as are resolved. The complexity of a behavioral situation is increased when the effects of a stimulus as complex as ionizing radiation are being studied. Radiation multiplies the number of variables which must be controlled and investigated, such as wave or particle form, dose rate, total dose, exposed area of the organism, time of testing, etc. At present some central and peripheral receptors for ionizing radiation have been tentatively and very generally hypothesized, e.g., olfactory bulb and vasculature. In physiological terms, however, receptors are defined to be specialized cells such as retinal rods and cones, cochlear hair cells, taste buds and olfactory hair cells, while in pharmacological usage, a receptor is any cellular component directly involved in the drug-cell combination initiating the drug action. According to these two definitions there is at present no known radiation receptor which makes any behavioral and physiological explanation of radiation-induced changes in performance more than highly tentative.

In general, performance in tasks which require a very low degree of drive tends to be disturbed by low doses of radiation (Brust-Carmona et al., 1966; Garcia et al., 1957, 1963; Hunt et al., 1964). On the

other hand, performance in those tasks which require a high level of maintained drive, such as discrimination in appetitive tasks (Kaplan et al., 1954a, b) or discrimination in shock-avoidance tasks (Melching et al., 1954; Kaplan et al., 1960; Riopelle, 1962) tends to persist even after supralethal doses. Although these results are based on performance by primates, some general application to other mammalian species would be expected.

In a recent study, Cheng (1968) compared the radiosensitivity of two appetitive tasks at identical levels of deprivation in rats under four different doses of cobalt-60 gamma irradiation. A dose-dependent increase of response latencies to conditioned stimuli was obtained three to four days after irradiation in a problem requiring a delay in response to a tone before water reward was given. In the other task, a differential paradigm in which response to a tone of one frequency (CS+) resulted in water reward, while response to a second tone (CS-) was unreinforced, discrimination performance, also monitored with latency measures, remained relatively stable.

The present study further investigated the effects of gamma irradiation on discriminatory performance when complicated or disrupted by a background of white noise. White noise of intensity greater than 85 decibels is an aversive stimulus for rats (Woods et al., 1967) and even below this level has disruptive effects on performance. The level of intensity was so chosen as to be high enough to interfere with performance and low enough to permit habituation. White noise of the same intensity as the auditory stimuli used as CSs could, on initial presentation,

produce either a startle or fear response which would interfere with any ongoing activity, or, it could interfere with only auditory discrimination of the two signals. This latter type of interference could confound the discrimination in the following ways: 1) by masking both CSs, which presumably could result in inhibition to CS+, disinhibition to CS-, or random lever pressing which would indicate deterioration of signal detection; 2) by masking only one of the CSs. If the CS- was masked, one would expect either complete inhibition which would result in errorless performance and would be difficult to distinguish from practice effects, or the subject could confuse the CS- for the CS+, resulting in an increase in the number of errors of commission (response instead of inhibition of response). If the CS+ was the only stimulus to be confounded by the white noise, one would expect an increase in the number of errors of omission (inhibiting response) as well as an increase in the response latency to CS+.

With these possible effects in mind, it was proposed that two studies be done: one to investigate the effects of gamma irradiation on performance after one week of habituation to white noise, and the second study to investigate the disruption in performance when both white noise and radiation were introduced during the same session. Since the effects of irradiation on discrimination only had been investigated (Cheng, 1968), another group was added to study the effects of white noise alone. Thus disruption due to white noise, habituation to it and any subsequent resensitization following irradiation, as well as the possible interaction of white noise and gamma irradiation on auditory frequency

discrimination were investigated.

Introduction

Recent reviews (Furchtgott, 1963, 1956; Gangloff et al., 1965; Kimeldorf et al., 1965; Lebedinsky et al., 1963; Livshits, 1960; Stahl, 1959, 1960) provide ample evidence of radiation effects on behavior. Many apparent contradictions, however, are cited, such as irreproducibility of results, changes in performance which one investigator interpreted as a learning deficit, another as an enhancement and still another as a change in motivational state.

Even with the preponderance of data demonstrating radiation-induced functional changes in both the central nervous system (CNS) and peripheral system, many reviewers and authorities are reluctant to detail possible radiation receptors, sites and modes of action (Gangloff et al., 1965; Buchwald et al., 1964; Kimeldorf et al., 1965; Stahl, 1962; Hug, 1962). That some of these functional changes are observed immediately after onset of exposure or immediately following sublethal doses, will be discussed in the following section. However, these manifestations are not always observed in experiments using diverse operant techniques and hence the rise of paradigm specificity. Paradigms using a delayed response appear to be very sensitive to radiation effects (Cheng, 1968; Riopelle et al., 1956; Nemenov cited by Stahl, 1960; Harlow et al., 1956; Brown et al., 1962; DiMascio et al., 1956). This has been verified in monkeys, rats and dogs. The interpretation of the effect varies: Cheng's study (1968) discusses only radiosensitivity in the performance of rats; Riopelle's

Harlow and Brown's results indicate enhancement in the performance of monkeys and DiMascio's indicate deficits in the delay response of dogs, which is similar to the findings of Burt et al. (1965) using rats.

Discrimination paradigms on the other hand, appear to be radio-resistant (Cheng, 1968; Riopelle, 1962; Kaplan et al., 1954; 1960; Melching et al., 1954) even at supralethal doses. However, such a paradigm was found to be very sensitive when one of the stimuli is the presence of ionizing radiation itself and the other is the absence of radiation (Garcia et al., 1957, 1962, 1963, 1964; Buchwald et al., 1964; Brust-Carmona et al., 1966).

Non-cognitive or emotional behavior, such as conditioned reflexes, orientation or arousal, or Pavlovian stereotype paradigms when subjected to radiation are usually found to be radiosensitive. One would expect any stress to be accompanied by changes in attention and emotionality. The non-specific stress of irradiation is more likely to be reflected in paradigms which respond to these changes in attention and emotionality. Discrimination paradigms do not appear to be sensitive to such changes. It may be that radiation-induced changes activated compensatory mechanisms that hide effects otherwise expected. The maintained performance of such tasks may become so stable that only a strong novel stimulus disrupts it and in this case, radiation does not appear to be this type of stimulus.

Historical Review

Functional effects of radiation on CNS. Changes in the morphology of the mature mammalian CNS generally require supralethal doses (usually

in the kiloroentgen range) (Gangloff et al., 1965; Kimeldorf et al., 1965; Stahl, 1960; Bachofer et al., 1960; Haymaker, 1962). Arnold et al. (1954a, b, c, d, e) found the primate hypothalamus to be particularly sensitive to high doses and rates. With 5000-7000 R, the entire hypothalamus was damaged. At 3000 R, the paraventricular and supraoptic nuclei were selectively affected, and at 1500 R, there was a lesser effect on the two nuclei. Ibrahim et al. (1967) and Haymaker et al. (1968) attributed the selective area of the CNS to vascular susceptibility rather than neuronal susceptibility.

Haymaker et al. (1958) using high intensity gamma-irradiation found a differential effect in the CNS of 67 monkeys. Gray matter of the cerebral cortex, the thalamus, supraoptic nuclei, pons and medullas showed the greatest damage. The decrease in humoral material in the neurohypophysis had been confirmed in the rat (Hopsu et al., 1960; Talanti et al., 1960) and in the supraoptic nuclei using X-irradiation (Hopsu et al., 1960).

Whole body X-irradiation with 600 R altered lever pressing rates for self stimulation in the posterior hypothalamus (Haley, 1962; Gangloff et al., 1959). Rats with low pre-irradiation rates, had a reduced rate on day 1, followed by a gradual increase to normal rates by the end of the 30 day test period. Those with an initial high rate had an initial increase and returned to normal levels during the next 17-19 days, then decreased below normal for the remainder of the period. This reversal in performance from pre-irradiation levels has been commented upon by several investigators engaged in different aspects of functional behavior.

Gangloff et al. (1960) observed an immediate increase in hippocampal activity of cats X-irradiated with 200 or 400 R whole body or head only. Since the effect did not occur with body only, it was thought to be centrally mediated. In addition to the hippocampal activity, changes were also noted in the thalamus and midbrain reticular system, but not in the cortex. After whole body or body only a decrease in threshold of the brain stem reticular formation was immediately observed, and was thought to be peripherally mediated. Control values returned three to four hours after irradiation. Behavioral alertness, EEG and a decrease in reticular activation threshold were observed after 400 R whole body. These effects were not pronounced in other than whole body and at the lower dose. The thalamic recruiting threshold increased later during the irradiation day and remained at that level for all Ss during the 16 post-irradiation test days.

These results were confirmed by later studies (Haley et al., 1962) using cats and (Monnier et al., 1961, 1962) rabbits. Reversal from pre-irradiated levels of hippocampal firing rates was observed in rabbits (Gangloff, 1962). This effect began immediately after 400 R X-irradiation.

In a preliminary study, Sams et al. (1964) using 1, 2, 10, 100 R observed spiking initiating in the cortex and spreading to subcortical structures in X-irradiated dogs. Cortical, hypothalamic and hippocampal EEG changes were dose and dose rate dependent. Although the onset of EEG changes was not given by the author, it was noted that 2 R delivered in six seconds produced a change after seven seconds.

Further evidence of radiosensitivity of the CNS is provided by the following studies. Desynchronization in rats and rabbits occurred almost immediately after onset of exposure (Grigoryev et al., 1958, 1960; Garcia et al., 1963). Hunt et al. (1962, 1963) and Cooper et al. (1964) found the threshold for desynchronization to be 0.1 and 0.2 R/sec. for rats. At this rate, habituation readily occurred. However, the arousal effect was sufficient to awaken sleeping rats. With intact rats, whole body X-irradiation was more effective than either head or body alone, which is consistent with Gangloff's finding in cats. Cooper et al. (1965) found that complete or extensive ablations of the olfactory bulbs greatly reduced sensitivity of EEG at 1.0 R/sec. head only or whole body at 0.5 to 2.5 R/sec. This has been confirmed by Hull et al. (1965). All of these findings illustrate prompt nervous reactions to ionizing radiation whether or not mediated by activation of peripheral receptors.

Functional effects of radiation on peripheral nervous system.

Barnes (1964) recorded electrical activity from the splanchnic nerve of anesthetized cats and rats during whole body X-irradiation (250 kv) using 32 R/min. Immediate reaction occurred by way of an increase in the number of high amplitude spike potentials and a slight increase in the average spike height. Normal levels reappeared 20-30 seconds after termination of radiation. Delitsyna (1960) also reported an increase in afferent activity after abdominal X-irradiation of 500 R in cats. However, the changes did not reach a maximum until 30-40 minutes after onset and were greatly dependent upon initial activity.

Although Barnes, in the above citation, found no effect on the

vagus nerve with X-rays at the stated intensity, Gasteiger et al. (1962) found that δ and β fibers of the cat vagus nerve were more sensitive than A fibers to β -Irradiation.

Lebedinsky (cited by Stahl, 1960) summarized radiation effects as "producing changes in respiration, pressor response, vascular permeability, gas exchange and general metabolism". However, results from his own laboratory indicate the effects to be unpredictable.

Gastrointestinal dysfunction is one of the most dominant effects and is usually accompanied by anorexia and loss of weight. The induction and degree of anorexia is species and dose dependent; dogs, hamsters, guinea-pigs and mice unlike monkeys, rats and rabbits have only slight reactions (Jones et al., 1959; Conard, 1956a; Ruch et al., 1962; Hunter et al., 1957; Gerstner, 1960; Smith et al., 1954). Carroll et al. (1961) found that anorexia was not detected when caloric intake was restricted before and after exposure. Thus the length and degree of deprivation will affect the degree of anorexia. Various investigators have remarked upon the apparent absence of radiation illness in their subjects (Brust-Carmona et al., 1966) after 306 R whole body of rats, (Livshits, 1956, 1958 cited by Livshits, 1960) after 300 to 400 R in dogs.

Gastric retention has been repeatedly confirmed by a number of investigators, using a variety of techniques (Goodman et al., 1952; Fenton et al., 1954; Baker et al., 1958; Schwartz et al., 1961; Swift et al., 1955). Jones et al. (1959) found the rate of transit reduced at onset of X-irradiation at 25 R/min. After 20 minutes, the transit in irradiated rats was 28 percent and in non-irradiated controls, transit

measurements were about 68 percent. With higher doses, gastric retention was obtained from exposure of tail and hindlimbs. The authors suggested the rapid onset of retention resulted from an autonomic trigger mechanism.

Exposure to X-rays promptly increased intestinal motility and muscle tone in rats, rabbits and cats, in vivo and in vitro (Swan, 1924; Conard, 1951; Craver, 1947; Hug, 1960). Conard (1951, 1952, 1956b) observed a decrease in cholinesterase activity on day one to four and increased levels of acetylcholine.

Initial hypotension has been observed from onset to one to three hours following exposure in cats, rats, monkeys and dogs (Swan, 1924; Weber et al., 1949; Brooks, 1956; Tikhonov, 1963). Blood transfused from irradiated rabbits (800 R) one and one-half hours post-irradiation produced a depressor effect in non-irradiated recipients (Painter et al., 1956). The same effect was observed in dogs by Gorizontov (cited by Kimeldorf et al., 1965). A reduction in blood pressure was reported in hypertensive patients receiving blood plasma obtained from patients undergoing radiotherapy by Zuppinger (cited by Kimeldorf et al., 1965). Haley et al. (1952) observed a biphasic reaction in whole body (600 R) X-irradiated rats. When blood, serum or plasma was injected into non-irradiated rats, a vasodepressor effect occurred the first week and a vasopressor effect during the second week. Philips et al. (1963) recorded aortic and tail blood pressures in rats receiving whole body (485 R) X-irradiation. During the 10 day test period, there was no change in central levels, but peripheral levels dropped within eight hours and recovered by day 2. A dose of 970 R was required to produce a slight drop in

aortic pressure within 24 hours.

Whole body X-ray (250 kvp) at 0.25 or 1.9 R/sec. was used by Hunt et al. (1964) to arouse sleeping normal, ophthalmectomized, and adrenal-ectomized rats. Increased heart rates were noted after 30 seconds exposure in all groups. The intensity of the reaction depended upon the rate rather than total dose. Subjects exposed to lower rates, habituated by 60 seconds and returned to sleep, those receiving the higher rate remained alert. In another study, these authors (1963) observed increased heart rate within one second using rates of 0.05-3.2 R/sec.

Changes in respiratory frequency and volume were noted by Pape et al. (1963) in patients exposed to 6 R X-rays at 1 R/sec., but not in sham exposures. Again the change was in the opposite direction to the pre-irradiation state. They suggested the effect to be the result of hypothalamic stimulation.

Increased cell permeability is a commonly observed reaction (Willoughby, 1960; Brinkman, 1962). Lebedinsky et al. (cited by Brinkman, 1962) found changes at low doses. Hug (1962) found temporary increases in permeability to Na^+ and K^+ which were a function of dose rate. Recovery occurred shortly after exposure. This finding was confirmed by Bachofer (1962), for peripheral nerves. Timiras et al. (1967), investigating the effect of radiation on the olfactory cortex, found shortened latencies in evoked potentials after 250 R and 500 R in rats. The data was averaged over ten day periods, thus the initial effect was not obvious. However, the authors remarked that the initial transient effect of 250 R was masked by this calculation. Several hypotheses were

suggested: increased permeability of the postsynaptic membrane, increased susceptibility of the neurotransmitter (which is thought to be acetylcholine), or increased rate of synthesis of the transmitter or decreased acetylcholinesterase. This study confirms the results of a previous one (Rosenthal et al., 1963) and extends the findings of Monnier et al. (1964) on the visual system i.e., retina, lateral geniculate body, visual cortex and limbic system.

In preliminary testing of the hypothesis that motivation induced by radiation was due to systemic humoral factors, Hunt et al. (1967) used pairs of rats either bound together physically or parabiotically. One member of each pair was irradiated with 360 R (24 R/min) while the other member was shielded. The pairs were given either preferred saccharin solution, or unflavored water 40 minutes following exposure. The following day's test resulted in a significant saccharin aversion for the shielded parabiotic member given the solution after the partner's exposure, but no aversion occurred in shielded parabiotics given water or physically tied together. Thus vascular anastomosis transmitted the substance(s) required for the conditioned aversion. From these studies it can be clearly seen that both the peripheral and CNS are radiosensitive, but what activates what is still a moot question.

Behavioral effects. Girden et al. (1933), using the method of limits, found a transient reduction of auditory thresholds of approximately 5.5 decibels in dogs exposed to 39-1000 R soft X-rays. Brodgen et al. (1937) using single exposures of 75 to 675 R found a 7-10 decibel increase in auditory sensitivity in dogs for 125, 1000 or 8000 cycles per

second. Appearance of the effect occurred from two to eight days and was inversely related to the dose, lasting for approximately two weeks for all doses. The effect was absent with hindquarter, thorax or hypophysectomized head only exposures. A similar effect occurred in pigeons given 500 R head X-irradiation. However, guinea-pigs, after an LD50 of 350 R whole body had increased thresholds of 2-25 decibels in the frequency range of 500 to 8000 cycles per second. Maximum loss of hearing occurred in the low frequency range as determined by cochlear sensitivity (Koslov, cited by Furchtgott, 1963; cited by Lebedinsky et al., 1963). Casey et al. (1963) exposed goats to 104 R of cobalt-60 gamma radiation and observed no effect. Murphy et al. (1961) found no change in rats after head X-irradiation.

Although general activity and certain manipulations were found to be depressed in primates after 300-500 R X-rays (Ruch et al., 1962; Harlow et al., 1956), the stability of the discrimination performance failed to be disturbed by irradiation of the supralethal doses. Frequently improvement has been reported (reviews by Furchtgott, 1963 and Kimeldorf et al., 1965).

Jarrard (1963) found no effect during the first week after 300 R in the performance of rats trained to jump within 10 seconds of the last response to avoid shock. Similar results were cited by Kimeldorf et al. (1965) using a 15 second interresponse interval and a dose of 1 kR. These studies are classed under discrimination rather than delay problems. The results would likely be different if the rats were trained to wait 10 or 15 seconds before jumping.